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REFERENCE STRAINS FOR THE SEROLOGIC GROUPS OF MENINGOCOCCUS (Neisseria meningitidis)

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Many variations in designation of serological groups of Neisseria meningitidis have appeared in the literature since the existence of such differences were first pointed out by Dopter in 1909. Confusion has resulted from inexact use of the terms "type" and "group", and from uncertainty as to the relation of these to each other, as well as from differences in terminology. The need for uniformity led to the organization of the Subcommittee on the Family Neisseriaceae of the International Committee on Bacteriological Nomenclature. This Subcommittee prepared a classification of N. meningitidis which conforms to the International Bacteriological Code of Nomenclature (1948). The preliminary report of the Subcommittee was given at the Fifth International Congress for Microbiology, Rio de Janeiro, Brazil, in 1950; a more complete report was presented at the Sixth International Congress for Microbiology in Rome, Italy, 1953. Both of these reports were published in the International Bulletin of Bacteriological Nomenclature and Taxonomy, 1954.

In the recommended classification the 4 main serological groups of the meningococcus are designated as A, B, C, and D. The relationship of these groups to other classifications is shown in tabular form by Branham (1953), and in a more condensed form in the 7th Edition of Bergey's Manual of Determinative Bacteriology.

Regardless of whether newer or older nomenclature has been employed, work with Neisseria meningitidis has been greatly handicapped by the lack of standard reference strains for the serological groups of this organism. Each center of activity in this field has been compelled to choose its own reference strains, or has obtained them from some other laboratory or center. Within major serologic groups individual strains of meningococcus may vary greatly in antigenic pattern. Some are "broad" enough to include all members of their group while others are so "narrow" that they fail to cover many strains, and might even be considered to form sero-types within the broad groups. Interest in the meningococcus is world wide and laboratories in scattered nations are concerned in its study. The use of uniform strains, broad enough to give good coverage to their respective serologic groups, yet sufficiently specific to avoid cross agglutination, would be of great advantage to workers making comparative studies. The International Committee on Bacteriological Nomenclature has suggested that "type" strains be chosen to represent the four major groups of meningococcus.

Since August 1, 1928, the author has received approximately 2200 strains of N. meningitidis for study, in addition to a number of strains of other Neisseriae. This period included the epidemics of 1928-30, of 1936-37, and of 1941-45. Although the majority of cultures came from acute infections during these three epidemics many were obtained from sporadic infections as well. The greatest number of all cultures came from the United States, but a few were from Canada, South America, Britain, and the European Continent. Not all of these meningogoccus cultures were conserved, though histories and records of study were kept for all. About 500 strains, representative of different periods and antigenic patterns, were preserved in a freeze-dried state. These cultures and records seemed to afford excellent material for a search for suitable strains to use as a beginning for establishment of a uniform basis of classification. Such a study was encouraged by both the Chairman of the International Committee on Bacteriological Nomenclature and by the Permanent Secretary for Medical Bacteriology.

A screening of this collection was done during 1956 and the early part of 1957. Group A strains were studied first,

then those of B, C, and D were taken in order.[1] Although it is not a meningococcus, N. flavescens was also included since it was involved in an epidemic, and this collection has the original strains (Branham, 1930).

In the preliminary screening of the collection, strains were studied in proups of 16. They were reconstituted from the dried state and primary cultures were made on blood agar slants and in semi-solid trypticase soy agar. The next day the growth was plated out on 5 per cent rabbit's blood agar and on the third day the 24-hour colonies were studied with a dissecting microscope and gram-stained smears prepared for microscopic examination for characteristic morphology.

Often 2 colony forms were apparent: one was a round raised and shining translucent cushion, and the other flatter and duller, but of similar translucency. As a rule neither form behaved like a "rough" colony, and they were not easily separable into cultures showing only one form. In such cases the whole culture was used for further study. With those strains that showed definitely "rough" and "smooth" colonies, "smooth" forms were chosen for further investigation.

After the study of colony form and of morphology, fermentation reactions were checked using a semi-solid, sugar-free agar base with indicator and 0.5 per cent of the desired carbohydrate added. All strains of meningococcus produced acid from dextrose and maltose only, though they varied in both rate of fermentation and in amount of acid produced. Sometimes the indicator change was very transient.

Antigenicity was checked by determining the presence of specific soluble capsular substance as shown by development of halos around implanted colonies on agar plates containing various dilutions (5 per cent, 2.5 per cent, and 1.25 per cent) of polyvalent anti-meningococcus serum (Pittman, Branham, and Sockrider, 1938, and Branham and Pittman, 1940). This polyvalent serum had been prepared previously in horses for diagnostic purposes and is not generally available. Halos were recorded as +, ++, +++, ++++. All smooth specific

[1]The author was aided by Mrs. Marion W. Grabowski and Mr. Donald B. Riggs, without whose help this intensive study would have been impossible.

strains of Groups A, B, and C produced halos of precipitate; Group D was not represented in the serum. All D strains available seemed to be completely rough. Halo production by Group B was less pronounced than by A and C, but all smooth strains did produce such rings to some extent. Halo formation proved to be one of the most valuable tests in screening out rougher, less specific, and less antigenic strains. Since capsule swelling has never been demonstrated with Group B strains of meningococci, this halo precipitation technique has a distinct advantage for screening cultures of this group.

All strains were tested for agglutinability with polyvalent and with homologous group sera, and also for cross agglutination with specific sera representing other groups. The specific group sera had been prepared at the National Institutes of Health and were those used routinely for diagnostic purposes. Their breadth and specificity had been established by extensive use. The regular tube agglutination technique was used with 4 to 6 dilutions of serum in 0.85 per cent NaCl solution buffered to pH = 6.8. Antigens were suspensions of 18 hour slant cultures in the buffered 0.85 per cent NaCl and standardized to contain approximately 1 billion (10^9, or a thousand million) meningococci per ml. The test was incubated at 37° C. in a water bath for 2 hours and then held in an icebox overnight before final reading. This method gives specific agglutination, whereas longer incubation is apt to result in "crossing".

On the basis of these preliminary observations a number of strains were selected for further study. Choice was made chiefly with two criteria in mind: breadth of coverage for strains of the same group, and specificity for the homologous group. Some strains were also selected for narrower characteristic serologic patterns which indicate the possibility of sero-types within the broad groups.

In order to determine the serological relation of these selected strains to each other and to their groups as a whole, it was necessary to prepare antisera for them. Rabbits were immunized by intravenous injection of 5-hour blood agar slant cultures. It was found best to begin with small amounts (i.e., 50,000,000 cells) of formalinized suspension, changing to living cultures as soon as practicable. No definite rate

of increase in size of doses can be prescribed, since individual response in rabbits varies widely, and the weight and condition of the rabbit is the safest guide. In general the dose schedule was as follows:

> 3 injections on successive days; rest 4 days.
> Repeat this for 2 more weeks. Rest one week.
> Repeat the above. Make trial bleeding from ear.

With Groups A and C a good agglutinating serum is usually obtained at the end of 8 weeks, though a second 6-week period of immunization is often advisable. With Group B several such courses are usually necessary. If immunization is long continued, the serum will "spread" and cross agglutination will occur as somatic agglutinins appear. A lower titered serum that is strictly specific is often preferable to one of high titer that cross agglutinates strains of other groups. When a satisfactory titer is obtained, the rabbits are bled from the heart.

An intensive serological study was made with the following strains chosen in the preliminary screening: 18 of Group A, 28 of Group B, and 27 of Group C. Since only 2 strains of Group D were in the collection, they were not studied in a similar fashion, though they were carefully checked separately for purity and specificity.

The serologic study of Group A strains presented few complications. The strains studied in this phase of the project included representatives from the epidemics of meningitis occurring from the period of World War I to the present, with several strains from sporadic cases. Group A is relatively homogeneous and differences among the strains are chiefly of degree of antigenicity, agglutinability, and halo intensity; i.e., those that were the best antigens produced agglutinating sera with broader coverage and higher titer.

Group C ranks next in order of homogeneity. More differences in serological pattern, in specificity, and in breadth of coverage have been found with Group C than with Group A.

Group B is relatively complex. Strains vary both qualitatively and quantitatively. With B more than with any other

group broad strains of recognized coverage are necessary for preparation of diagnostic sera. If agglutinating sera made from "narrow" members of this group are used for diagnostic purposes some strains of B are apt to be missed. There is considerable evidence for "sero-types" within Group B though there is always overlapping.

There is some evidence for the occurrence of "types" within all groups of meningococcus. Gordon and Murray (1915), by absorption of agglutinins, found Types I and III within the broad Group A. This is the most homogeneous group with the simplest antigenic composition. It is likely that the more complex Groups B and C would yield "types" if painstaking studies were made.

Some of the complications arising in a serological study of meningococcus may well be mentioned here. Some strains acquire a tendency to agglutinate spontaneously. This is usually due to the occurrence of rough forms in the culture and is remedied by plating out, and picking and comparing several smooth colonies. A similar procedure may be employed when a strain habitually does not agglutinate well. Individual colonies vary considerably in agglutinability. Cross agglutination between groups, likewise, may be due to the presence of rough organisms in the culture, since rough meningococci have lost their specific capsular substances and the somatic antigens react nonspecifically. Antisera agglutinate nonspecifically if: (1) they are prepared by the use of rough antigens; or (2) if immunization of the rabbits is carried on too long. The sensitivity of an agglutination test can be increased by (1) lowering the pH of the salt solution used; or (2) slightly decreasing the density of the antigen suspension. The stability of a suspension can be increased by lowering the concentration of electrolyte, i.e., from 0.85 per cent NaCl to 0.5 per cent.

On the basis of the studies made, strains of meningococci have been chosen to form a special collection. For each group there is suggested a "type strain", or more properly, a "neotype", to serve as a reference strain for the group, and two other similar strains as "runners-up" in case a substitution should seem desirable. These are chosen on the basis of specificity, antigenicity, and breadth of coverage for their group. In addition there are a few other selected

strains which represent special serological patterns, possible sero-types within the broad groups, individual outbreaks of meningitis, and strains of special historial interest. These strains are listed in the appended tables.

Although N. flavescens is not a meningococcus it was also studied since it is the only other member of the genus that has been known to be involved in an epidemic of meningitis (1930). Available strains were studied in the same manner as described above for the meningococcus except that new antisera were not prepared for them since good agglutinating serum was already on hand. This species of Neisseria ferments no carbohydrates, produces a golden yellow pigment (greenish yellow on Loeffler's blood serum medium) and agglutinates specifically. Six strains of N. flavescens are included in this special collection. These are listed at the end of the table below.

The strains in this special collection have been dried from a frozen state and sealed in vacuo. As long as the supply lasts laboratories and qualified individual workers may obtain them on request from the Division of Biologics Standards, National Institutes of Health, Bethesda 14, Maryland, U.S.A. They are also being deposited in the American Type Culture Collection, 2112 M Street, NW., Washington, D. C., U.S.A.

It is hoped that requests will specify which strains are desired; i.e., whether the neotypes only, or others for special work, so that specific needs of workers may be met.

SUMMARY

Neotype strains have been suggested to represent the major serologic groups of meningococci: A, B, C, and D. Two other similar strains of each group have been selected as "runners-up" for these. It is hoped that these will be useful as reference strains in classification and in comparative studies.

These reference strains, together with a few others which represent special serologic patterns, or which have historical significance, form a special collection which is available to workers in this field.

Serogroups and Strains of Neisseria meningitidis and of N. flavescens

Serogroup	NIH* Strain number	Date received at NIH	Date and place isolated	Received from	Source	Labelled	Comment
A	M1027	4/12/37	4/37, Municipal Contagious Dis. Hospital, Chicago, Ill.	Dr. C.P. Miller Univ. of Chic.	Spinal fluid	"21" A fatal case	Neotype strain for Group A. Neotype strain for species.
A reliable strain. Makes good agglutinating serum for Group A. Has a tendency to agglutinate spontaneously at times. This can be easily overcome by plating out and testing individual colonies for stability.							
A	M962	1/12/37	1/37, Baltimore, Md.	City Health Dept. Baltimore, Md.	Spinal fluid	"S-3032-S"	A good "runner-up" for neotype strain of Group A.
An epidemic strain from outbreak of 1936-37.							
A	M1894	7/30/43	7/43, Washington, D.C.	Gallinger Hospital (now D.C. General)	Spinal fluid	"Bernard Bice"	A good "runner-up" for neotype strain of Group A.
An epidemic strain of World War II.							
A	M947	12/10/36	12/36, Boston, Massachusetts	Antitoxin and Vaccine Lab., Jamaica Plain	Spinal fluid	"Whitehouse"	A good specific strain.
An example of the strains involved in epidemic of 1936.							
A	M973	1/22/37	1/37, Boston, Massachusetts	Antitoxin and Vaccine Lab., Jamaica Plain		"Horan (2)"	Specific, antigenic.
Another typical epidemic strain.							
A	M1036	4/26/37	4/37, Wilmington, N.C.	James Walker Memorial Hospital	Spinal fluid	"James Scott"	Specific.
Another Group A strain typical of those in epidemic of 1936-37.							

*NIH refers to the National Institutes of Health, Bethesda, Maryland

Serogroups and Strains of Neisseria meningitidis and of N. flavescens Con't.

*NIH refers to the National Institutes of Health, Bethesda, Maryland

Serogroups and Strains of Neisseria meningitidis and of N. flavescens Con't.

Serogroup	Strain number	Date received at NIH	Date and place isolated	Received from	Source	Labelled	Comment
A	M1157	2/10/40	2/40, Wilkesbarre, Penn.	Dr. Edward S. Pugh Kirby Health Center	Spinal fluid	"Stella Pendock"	An early World War II strain. During epidemic.
A	M2055	1/31/46	1/46, Washington, D.C.	D.C. General Hospital	Spinal fluid	"Robert Jones"	A reliable specific strain.
Not from an epidemic but from a sporadic postwar case.							
A	M2184	1/6/56	1/56, Helena, Montana	Miss E. Kuhns Montana State Lab.	Blood	"Clarence Bird Rattler"	Most recent A strain.
From sharp outbreak on Blackfoot Indian Reservation, aborted by giving sulfadiazine to entire community.							
A	M57	6/8/17	World War I	Rockefeller Inst., New York	Spinal fluid	"Irregular, 30, Isadore"	Still a good strain.
A very old strain representative of Gordon and Murray's Type III. Also Wollstein's "Irregular" (1914). Used as a reference strain for "Type III" for years in USA. Evans' Tropin group "R" (1920). A good representative of Group A. Kept both for historic reasons and because it is still a good strain.							
A	M423	6/29/31	1915, London, England	Dr. W.M. Scott	Spinal fluid	"Myott"	Isolated by Gordon and Murray in 1915.
Preserved as "Type III" (Gordon and Murray) in National Type Culture Collection (London), but not the original standard "Type III". Considered degenerate by Scott and certainly "rough" now, though some "halo" production indicates presence of some specific cells. Kept for historic reasons.							
A	M331	5/1/30	5/30, Memphis, Tenn.	Dr. A.D. Dulaney Univ. of Tenn.	Spinal fluid	"30-7"	Narrow A.
Used for a long time as a reference strain for "Type I" of Gordon and Murray; sufficiently narrow not to cross agglutinate with "Type III" of Gordon and Murray. No longer used as reference strain but included here because of its history.							
A	M123	1918	-----	Rockefeller Inst., New York	Spinal fluid	"Rockefeller Inst., 85"	A very old strain.
Long used at NIH as reference strain for "Type I" of Gordon and Murray. Evans' Tropin group "R" (1920). Now rough. Kept for historic reasons.							

Serogroups and Strains of Neisseria meningitidis and of N. flavescens Con't.

Serogroup	Strain number	Date received at NIH	Date and place isolated	Received from	Source	Labelled	Comment
B	M2092	1/8/48	1/48, Boston, Mass.	Antitoxin and Vaccine Lab., Jamaica Plain	Spinal fluid	"Linda Cunningham"	Neotype strain for Group B.
This strain is a good immunizing agent and makes good broad agglutinating serum that includes all B's, yet does not cross with other groups. It agglutinates itself well, but is agglutinated rather poorly by many other B sera.							
B	M2091	10/29/47	10/47, Boston, Mass.	Antitoxin and Vaccine Lab., Jamaica Plain	Nose	"Elaine Street"	A "runner-up" for neotype of Group B.
The only shortcoming of this strain is a tendency to become "rough" and to agglutinate spontaneously. Otherwise it is an excellent antigen, makes a good broad diagnostic serum which agglutinates specifically. Occasionally necessary to plate out and test picked colonies for stability.							
B	M997	2/20/37	2/37, Baltimore, Maryland	City Health Department, Baltimore, Md.	Spinal fluid	"S-3250-L"	A "runner-up" for neotype of Group B.
This strain is a slow immunizing agent, but will make an excellent agglutinating serum if immunization is continued long enough. The serum is both broad and specific.							
B	M1385	4/23/42	4/42, Washington, D. C.	Children's Hospital	Blood	"Eugene McKinney"	A broad strain.
This strain makes a serum that is fairly inclusive for Group B, but it has a tendency to overlap other groups and to cross agglutinate to some extent.							
B	M1180	6/6/40	6/40, Syracuse, N. Y.	Dr. A. C. Harris	Spinal fluid	"Margaret Sarafin"	A relatively narrow B strain.
Has characteristic antigenic pattern resembling M2176 and M2189.							
B	M1206	2/20/41	2/41, Phillipsburg, Pennsylvania	Dr. Vernon Nisbet, Department of Health Philadelphia	Spinal fluid	"Anna Potella 18 months' old"	Fairly narrow B strain.
Has characteristic serologic pattern unlike M1180, M2176, M2189.							

Serogroups and Strains of Neisseria meningitidis and of N. Flavescens Con't.

Serogroups and Strains of Neisseria meningitidis and of N. Flavescens Con't.

Serogroup	Strain number	Date received at NIH	Date and place isolated	Received from	Source	Labelled	Comment
B	M1776	4/7/43	4/43, Washington, D. C.	Army Medical School (now Walter Reed Army Medical Center)	Naso-pharynx	"Burroughs, No. 4324"	Fairly narrow B strain.
From Camp Shelby, Mississippi, during World War II.							
B	M1992	4/1/44	4/44, Chicago, Illinois	Dr. C. P. Miller University of Chicago	Carrier	"Mn 116"	A narrow B strain.
Of a serological pattern designated as "Y" by Dr. Miller.							
B	M2176	3/25/54	3/54, Washington, D. C.	District General Hospital	Spinal fluid	"Ellen Ross, 18 mos. old"	A narrow B strain, related to M1180, M2189.
Isolated after death of child with Waterhouse-Friderichsen Syndrome.							
B	M2189	1/20/56	1955, Oslo, Norway	Dr. Kaare Jyssum, Rikshospitalet, Oslo, Norway	-----	"M10"	A very narrow specific B strain.
Not agglutinated by all Group B sera, but is agglutinated by those that are broad. Called "type N" by Jyssum (1956). Related to M1180 and M2176.							
B	M55	1917	----------	Rockefeller Institute, N. Y.	Spinal fluid	"Regular str. No. 1"	A very old strain.
This strain dates back to World War I. It was one of Dr. Wollstein's strains. Was long used at NIH as a reference strain for Gordon-Murray's "Type II". Also an example of Evans' Tropin Group "S" (1920). Now chiefly of historic interest as it has become "rough".							
B	M963	1/15/37	1/37, Toronto, Canada	Dr. Geoffrey Rake, Toronto, Canada	-----	"Herrington"	Old reference strain.
Used for years for testing therapeutic serum. It had a persistent mouse virulence and specificity, but was a very poor antigen for immunization. Is included for research purposes.							

Serogroups and Strains of Neisseria meningitidis and of N. Flavescens Con't.

Serogroup	Strain number	Date received at NIH	Date and place isolated	Received from	Source	Labelled	Comment
C	M1628	11/25/49	11/49, Hartford, Conn.	Dr. Friend Lee Mickle, Department Public Health	Spinal fluid	-----	Neotype strain for Group C.
A broad strain serologically for strains of Group C and also specific for the group. Makes a good "typing serum".							
C	M1054	6/3/37	6/37, Baltimore, Maryland	City Health Dept., Baltimore, Maryland	Spinal fluid	"S-3766-S"	A "runner-up" for neotype of Group C.
The original "II alpha", studied in NIH Laboratories (1942).							
C	M1166	3/4/40	3/40, Buffalo, New York	Miss Sophia Cohen, N. Y. State Dept. of Health Lab., Albany	Spinal fluid	"No. 3855"	A "runner-up" for neotype of Group C.
The first strain of this group to be reported in the USA. Described by Cohen, 1940.							
C	M1170	3/21/40	3/40, Syracuse, New York	Dr. A. C. Harris, Department of Health	Spinal fluid	"Patient David Essig"	A narrow strain.
This strain is typical of a tight little group within the broad Group C. Has characteristic serologic pattern. Is useful in clarifying obscure relationships in Group C.							
C	M1375	4/10/42	4/42, Boston, Mass.	Thorndyke Labs., Boston, Mass.	Spinal fluid	"Lawrence"	A broad strain.
Typical of the "II alpha" strains found during World War II.							
C	M1875	6/4/43	6/43, Camp Blanding, Florida	Army Medical School Washington, D. C.	Blood	"Norman Prague, No. 6106"	An example of strains of this group found in World War II.

Serogroups and Strains of Neisseria meningitidis and of N. flavescens Con't.

Serogroups and Strains of Neisseria meningitidis and of N. flavescens Con't.

Serogroup	Strain	Date received at NIH	Date and place isolated	Received from	Source	Labelled	Comment
C	M1883	6/4/43	6/43, Camp Shelby, Miss.	Army Med. School, Washington, D. C.	Spinal fluid	"Ben Tcma-shiro 6346"	Another example of Group C from World War II.
C	M2105	8/13/51	Dordogne, France	Dr. Jean Chevé, Pasteur Inst. Annexe	----	"C107"	Representative of old French classification C, but not an old strain.
Represents classification of Nicolle, Debains, and Jouan, 1918.							
C	M2108	8/13/51	Dordogne, France	Dr. Jean Chevé, Pasteur Inst. Annexe	----	"C113"	Another representative of the French C.
C	M2129	2/4/53	2/53, Washington, D.C.	Dr. Grace Guin Children's Hosp.	Blood	"Phyllis Blue, age 6"	Waterhouse-Friderichsen syndrome.
C	M2159	5/9/53	Paris, France	Dr. Thibault, Pasteur Inst.	----	"N. meningitidis type C 52139"	Sent by Pasteur Institute as example of their C.
Old French classification reported by Nicolle, Debains, and Jouan, 1918.							
D	M158	8/15/28	8/28, Chicago, Ill.	Chicago Dept. Health	Spinal fluid	"37A"	Neotype of Group D.
From an epidemic in which all serological groups were found. Quite a number of cases due to Group D, but only in Chicago; elsewhere Groups A and B caused 100 per cent.							
D	M60	11/18/19	1917	Rockefeller Institute	Spinal fluid	"Para strain 81"	A very old "Type IV".
"Para strain 81" refers to an old Rockefeller Inst. classification (1914). This strain used as reference strain for Type IV (Gordon and Murray) for many years. Evans' Tropin group "Z" (1920). Now a "rough" strain but included here for historic reasons.							

Serogroups and Strains of Neisseria meningitidis and of N. flavescens Con't.

Serogroup	Strain number	Date received at NIH	Date and place isolated	Received from	Source	Labelled	Comment
	N155	6/11/28	6/28, Chicago, Ill.	Chicago Dept. of Health	Spinal fluid	34A	Neisseria flavescens
This is the actual type strain of N. flavescens n. sp., found in the spinal fluid of 28 cases of meningitis in Chicago during the widespread meningococcus epidemics of 1928-30. Reported 1930.							
	N156	6/11/28	6/28, Chicago, Ill.	Chicago Dept. of Health	Spinal fluid	35A	Neisseria flavescens
Similar to the type strain, N155, and with same history.							
	N157	8/15/28	8/28, Chicago, Ill.	Chicago Dept. of Health	Spinal fluid	36A	Neisseria flavescens
Similar to the type strain, N155, and with same history.							
	N159	8/15/28	8/28, Chicago, Ill.	Chicago Dept. of Health	Spinal fluid	38A	Neisseria flavescens
Similar to the type strain, N155, and with a similar history.							
	N204	3/28/29	3/29, Chicago, Ill.	Chicago Dept. of Health	Spinal fluid	41A	Neisseria flavescens
	N208	3/22/29	3/29, Chicago, Ill.	Chicago Dept. of Health	Spinal fluid	48A	Neisseria flavescens

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THE NOMENCLATURE AND TAXONOMY OF THE PROTEUS AND PROVIDENCE GROUPS

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The classification of the genus Proteus given by Rustigian and Stuart (1943, 1945) has gained wide acceptance and is now in use in many parts of the world. In recent years, however, several suggestions and proposals have been made regarding the taxonomy and nomenclature of the Proteus group and also of the Providence group. Kauffmann (1953, 1954, 1956) proposed a classification of this general group of microorganisms in which the genus Proteus was limited to two species, P. vulgaris and P. mirabilis. P. morganii and P. rettgeri were excluded from the genus on the basis of biochemical differences and were named Morganella and Rettgerella, respectively. Providence was maintained as a separate entity under the name Providencia. Proom and Woiwod (1951) excluded P. rettgeri from the genus Proteus and Proom (1955) advised the establishment of a new genus to include both P. rettgeri and Providence. Buttiaux et al. (1954) proposed the name Proteus stuartii for members of the Providence group and suggested that they be included in the genus Proteus under this designation. Shaw and Clarke (1955) disagreed with Kauffmann's division of the Proteus group and proposed the name Proteus inconstans for members of the Providence group, which they added to the genus Proteus as a fifth species. Cowan (1956) also adopted P. inconstans. Several writers (Borman et al., 1944, Buttiaux et al., 1954, and others) have included both P. vulgaris and P. mirabilis in one species called P. vulgaris by some and Proteus hauseri by others. Lastly, it should be mentioned that Peluffo (1953) reported that the flagella of P. vulgaris, P. mirabilis, and P. rettgeri strains all had the same spiral size, while those of P. morganii cultures had a definitely longer one, and this investigator suggested that the taxonomic position of P. morganii might be rediscussed in the light of this finding. This fundamental observation of Peluffo's is ancillary evi-

dence in favor of the separate status of P. morganii. However, the writer believes that in view of the present state of knowledge of the subject, the observation might better be construed as evidence in favor of separate species status, rather than separate generic status.

Stuart et al. (1946), Buttiaux et al. (1954), Shaw and Clarke (1955), and others have pointed out the resemblance of Providence group cultures to members of the genus Proteus. Further, the fact that members of both groups produce phenylpyruvic acid from phenylalanine lends additional support to the view that the two groups are closely related. However, the writer does not believe that this fact warrants actual inclusion of members of the Providence group in the genus Proteus, as the genus is presently constituted. As pointed out by Ewing et al. (1954), members of the Providence group resemble P. morganii and P. rettgeri in many respects but are urease negative. However, members of the Providence group differ markedly from cultures of P. vulgaris and P. mirabilis in certain fundamental characteristics such as failure to liquefy gelatin and failure to produce large amounts of hydrogen sulfide. Also, Providence group strains do not swarm in the manner often observed in cultures of P. vulgaris and P. mirabilis, although many Providence cultures can be induced to swarm under certain conditions (Stuart et al., 1946). The Providence group is composed of two principal biogroups, the members of which are readily distinguishable in all but a very few instances (Ewing et al., 1954). The first of these biogroups resembles P. morganii and the second, P. rettgeri. Thus, it may be seen that members of the Providence group are similar to two species of the genus Proteus but quite different from the other two (P. vulgaris and P. mirabilis). Therefore, it is the opinion of the writer that the Providence group should not be incorporated into the genus Proteus as it is now constituted. If the modern classification of the genus Proteus (Rustigian and Stuart, 1946) is retained, then both the Proteus and Providence groups should be maintained as separate entities within a larger principal division (or tribe), which may be called the Proteus-Providence division or, if a tribal designation is required for nomenclatural purpose, the tribe Proteae. In this case the generic term for the Providence group would be Providencia (Kauffmann, 1953) and the proper specific name would appear to be Providencia inconstans.

The work of Proom (1955), who regarded the Providence group as nutritionally homogeneous and different from Proteus in certain nutritional aspects, tends to support the concept of a separate Providence group. However, it should be said that not enough is known about fundamental nutritional differences to assess their taxonomic value at present. Undoubtedly, nutritional studies eventually will be made with large numbers of cultures belonging to each of the groups of Enterobacteriaceae and the results of such investigations may prove to be of value in taxonomy. Proom and Woiwod (1951) and Proom (1955) advocated the exclusion of P. rettgeri from the genus Proteus because P. rettgeri cultures fermented mannitol, produced only traces of hydrogen sulfide (as measured by a lead acetate paper method), failed to produce gas from glucose, failed to produce volatile amines, and differed from other Proteus species with respect to nicotinic acid requirements. The writer regards this proposal (per se) as untenable, since at least 10 per cent of P. rettgeri strains produced gas from glucose (Rustigian and Stuart, 1945, and author's unpublished data). Also, it may be noted that one of the 10 P. rettgeri cultures examined by Proom and Woiwod (1951) produced gas. The fact that three Proteus species fail to ferment mannitol while P. rettgeri strains do so does not seem to be a valid reason for removal of the latter species from the genus, since mannitol positive and mannitol negative species and varieties are known to exist in certain other recognized genera and species of Enterobacteriaceae, e.g. Shigella and Shigella flexneri. Furthermore, it is known that certain cultures otherwise like P. rettgeri fail to produce acid from mannitol (unpublished data). As regards tests for hydrogen sulfide production Proom (1955) states that "it seems clear that the conditions of the test can be arranged to produce almost any desired effect." It is probable that all Enterobacteriaceae produce at least small amounts of hydrogen sulfide from organic or inorganic substrates if sufficiently sensitive methods are used to detect its presence, but the writer believes that in this instance the most sensitive method is not necessarily the best for taxonomic purposes. Rather, it is felt that for a hydrogen sulfide test to be of value in taxonomic work within the family Enterobacteriaceae, it should be poised at a certain level of sensitivity. TSI agar, or a medium of similar composition but without lactose and sucrose, serves this purpose admirably and may be recommended because it gives results comparable to classical

descriptions and permits easy primary group differentiation within the family. If a lead acetate paper method is used it should be employed as it is with Brucella cultures, i.e. in a semi-quantitative way. The papers should be replaced with new ones each day for four days and records kept as to weak, moderate, or marked hydrogen sulfide production. If this is done, the results obtained are comparable to those obtained with TSI agar, or other medium of similar sensitivity, except that cultures giving weakly positive paper tests may be negative in TSI agar. Nicotinic acid requirements and other criteria of similar nature need to be studied further before their importance and value in taxonomy can be accurately assessed. For these reasons the writer cannot agree to the exclusion of P. rettgeri only from the genus Proteus. Nor can he agree to the suggestion of Proom (1955) that a new genus should be created to include only P. rettgeri and the Providence group.

The above-mentioned considerations regarding the taxonomy and nomenclature of the Proteus and Providence groups seem to resolve themselves into the following questions:

> Should the modern classification of the genus Proteus be retained, or should the genus be split, and if the genus is to be divided, how should the division be made?

Something may be said in favor of each of the proposals and suggestions made by Kauffmann (1953, 1954, 1956), Proom and Woiwod (1951), Proom (1955), and Shaw and Clarke (1955). Also, certain aspects of each may be adversely criticized. It is possible that a compromise may be desirable in this particular situation. For example, two genera might be recognized, the first of which would be Proteus and would include P. vulgaris and P. mirabilis as distinct species, since it appears that there are sufficient differences between these to warrant species status for each. The second genus might be called Morganella, (Fulton, 1943) since this name appears to have priority, and would include Morganella morganii, M. rettgeri, and M. inconstans. (Table 1.) If it is granted that there are sufficient differences between P. vulgaris and P. mirabilis on the one hand, and P. morganii, P. rettgeri, and the Providence group on the other, to warrant generic status for each, then the above-mentioned compromise

Table 1

Biochemical reactions given by members of the Proteus and Providence groups
(Compromise schema of nomenclature and taxonomy)

	Proteus		Morganella			
	P. vulgaris	*P. mirabilis*	*M. morganii* (*P. morganii*)	*M. rettgeri* (*P. rettgeri*)	*M. inconstans* Biogroup 1	(Providence) Biogroup 2
Gas from glucose	+	+	+	d	+	-
Lactose	-	-	-	-	-	-
Sucrose	+	x	d	d	d	d
Mannitol	-	-	-	+	-	d
Dulcitol	-	-	-	-	-	-
Salicin	+	d	-	d	-	-
Adonitol	-	-	-	+	+	-
Inositol	-	-	-	+	-	+
Indol	+	-	+	+	+	+
Methyl red	+	+	+	+	+	+
Voges-Proskauer	-	d	-	-	-	-
Simmons' citrate	d	d	-	+	+	+
H_2S (TSI agar)	+	+	-	-	-	-
Urease	+	+	+	+	-	-
Gelatin	+	+	-	-	-	-
KCN	+	+	+	+	+	+
Motility	+	+	+	+	+	+
Lysine decarboxylase (ninhydrin)	-	-	-	-	-	-
Phenylalanine deaminase	+	+	+	+	+	+

+, positive 1 or 2 days. x, late and irregularly positive. -, no reaction. d, different biotypes

might be satisfactory to those who would divide the genus Proteus. In this way, biochemically similar species are grouped together and the species rank of each of the five species is maintained. P. morganii and P. rettgeri are placed in the same genus because it is felt that there are not enough known differences between them to warrant their being given separate generic status. Members of the Providence group are given separate status as a species and certainly this is necessary at this time for practical reasons, if for no other. However, it is possible that future studies may indicate that members of the Providence group should be considered as urease negative varieties of either P. morganii (M. morganii) or P. rettgeri (M. rettgeri), according to reactions given by individual strains.

The compromise mentioned above is presented for consideration along with suggestions already made and proposals which undoubtedly will be made in the future. Since it may be decided that the classification of the genus Proteus given by Rustigian and Stuart (1943, 1945) should be retained, that classification should remain in use until such time as agreement on the taxonomy and nomenclature of the group is reached. Until that time the Providence group should be maintained as a separate entity.

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REPORT OF THE ENTEROBACTERIACEAE SUBCOMMITTEE OF THE NOMENCLATURE COMMITTEE OF THE INTERNATIONAL ASSOCIATION OF MICROBIOLOGICAL SOCIETIES

(To be presented to the International Committee on Bacteriological Nomenclature at the VII International Congress of Microbiology, 1958.)

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ENTEROBACTERIACEAE

Definition and Principles of Classification

The family Enterobacteriaceae is composed of Gram-negative rod-shaped bacteria, which are motile with peritrichous flagella or nonmotile. They ferment glucose rapidly with or without gas production, reduce nitrates to nitrites, and grow on ordinary media.

The principles of classification given in the 1950 and 1953 Enterobacteriaceae Reports[1] are reaffirmed. However,

[1] International Bulletin of Bacteriological Nomenclature and Taxonomy, 4:1-94. 1954.

these principles are restated here for purposes of emphasis, clarity, and convenience.

The family Enterobacteriaceae is made up of a series of interrelated bacterial types and segregation of these into groups often is difficult because the transition from group to group is gradual and intermediate forms exist in all instances. However, within the family there are dense centers of population composed of biochemically homogeneous bacteria, which also show a high degree of serological relationship, and it is these centers that form the biochemical groups mentioned herein.

In turn, the principal biochemical groups within the family are divided into subgroups on the basis of serology, on certain biochemical reactions, or by a combination of both methods. Generally, the groups are divided into subgroups on the basis of the O antigens of the bacteria, as in the case of the Salmonella, Arizona, Citrobacter, Escherichia, Providence, and Serratia groups. The O antigen subgroups are further divided into serotypes, which are characterized by the possession of particular O antigen fractions, K (sheath or capsular) antigens, or H (flagellar) antigens. Further, some serotypes may be composed of two or more biotypes. Some groups are divided into subgroups by means of a combination of biochemical and serologic characteristics. For example, the Shigella group is composed of four subgroups, which are differentiated by means of the serological and biochemical characteristics of the serotypes contained in each subgroup. In the Klebsiella group, subdivision generally is made directly into serotypes by means of capsular antigen determination, although several O antigen groups are known. Also, three principal biochemical subgroups are recognized within the Klebsiella group; these correspond to the species Klebsiella pneumoniae, K. rhinoscleromatis, and K. ozaenae. At present, the Proteus group is subdivided on the basis of biochemical reactions alone, although an antigenic schema has been established for a portion of the group (Proteus vulgaris and P. mirabilis). Certain other principal biochemical groups such as the Cloaca (Aerobacter) and Hafnia groups have not been subdivided.

Some serotypes within certain groups may be composed of two or more biotypes, as is the case, for example, with cer-

tain salmonellae. In some instances, particular biotypes or serotypes have been given specific names (e.g., Salmonella typhi or K. ozaenae) that are generally recognized. However, it should be emphasized that all of the serotypes within the groups are not regarded as species. On the contrary, only one species of Escherichia is recognized but there are many E. coli serotypes and four species are recognized within the Shigella group, each of which contains a number of serotypes, etc.

A report on the Proteus group is not presented at this time. Several proposals have been made regarding the nomenclature and taxonomy of the Proteus and Providence groups and these are being studied by the Subcommittee. Until agreement is reached on this subject, the Subcommittee recommends the continued use of the classification of the Proteus group devised by Rustigian and Stuart (1945)[1] as in the appended table of biochemical reactions, and the continued maintenance of the Providence group as a separate entity.

Also, no report on the Alkalescens-Dispar group is presented since its status largely remains unchanged. This group actually is composed of anaerogenic nonmotile E. coli biotypes that belong to various E. coli O antigen groups and may be reported as such if desired.

Since the majority of the Subcommittee members prefer the terms "groups" and "types" to "genera" and "species", only common names are used in the report. However, the group names may be used in a generic sense and with generic connotation if desired.

The definitions and descriptions of the various groups of Enterobacteriaceae are given in the following pages. Attention should be directed to the fact that in those groups in which the bacteria are described as motile, nonmotile variants may occur. The key to the symbols used in the definitions of the groups, and in the appended table of biochemical reactions, is as follows:

+	positive reaction, 1 or 2 days
x	late and irregularly positive
-	negative, no reaction

[1] Jour. Bact. 49:419. 1945.

d	different biochemical types
(+)	gelatin liquefied slowly, urea hydrolyzed slowly, or hydrogen sulfide produced slowly and in small amounts

The term phenylalanine deaminase employed in the definitions and the table refers to the enzyme system that is responsible for the oxidative deamination of phenylalanine to phenylpyruvic acid.

A list of "Recommended biochemical methods for group differentiation within the Enterobacteriaceae", which has been approved by the majority of the Subcommittee members, is appended to this report. It is suggested that these methods be employed in the interests of uniformity and better taxonomy.

THE SALMONELLA GROUP

(Sixth Report)

Salmonella Definition

The Salmonella group consists of motile or nonmotile bacteria that conform to the definition of the family Enterobacteriaceae, and have the following biochemical characteristics:

Gas from glucose	+
Lactose	-
Sucrose	-
Mannitol	+
Dulcitol	+
Salicin	-
Adonitol	-
Inositol	d
Indol	-
Methyl red	+
Voges-Proskauer	-
Ammonium citrate	+
Hydrogen sulfide	+
Urease	-
Gelatin liquefaction	-
Growth in KCN medium	-

Phenylalanine deaminase	-
Sodium malonate	-

Newly Recognized Serotypes

A list of Salmonella serotypes recognized by the Subcommittee in the interval between the publication of the 1953 Report and December 31, 1957 will be published as a supplement to this report. These serotypes may be inserted into the Kauffmann-White Schema (see Fifth Salmonella Report, International Bulletin, 4:54-72. 1954. and Fourth Salmonella Report, ibid., 4:11-17. 1954.

THE ARIZONA GROUP

(Third Report)

Arizona Definition

The Arizona group consists of motile bacteria that conform to the definition of the family Enterobacteriaceae, and have the following biochemical characteristics:

Gas from glucose	+
Lactose	+ or x
Sucrose	-
Mannitol	+
Dulcitol	-
Salicin	-
Adonitol	-
Inositol	-
Indol	-
Methyl red	+
Voges-Proskauer	-
Ammonium citrate	+
Hydrogen sulfide	+
Urease	-
Gelatin liquefaction	(+)
Growth in KCN medium	-
Phenylalanine deaminase	-
Sodium malonate	+

The second report is affirmed. In the interim additional O and H antigens were recognized and many new serotypes established. At present, 32 O groups, 37 H antigens and 144 serotypes are known. The KCN test of Moeller (1) and the ninhydrin test of Carlquist (2) have been used to differentiate Arizona from Citrobacter strains. The Arizona group fails to grow in KCN medium and is ninhydrin positive, whereas the results of these tests are the reverse with the Citrobacter strains. Shaw (3), Kauffmann and Petersen (4) and Ellis, Edwards and Fife (5) have utilized organic acids[1] to differentiate typical Salmonella, Citrobacter, and Arizona strains in the following manner.

	Mucate	D-Tartrate	Citrate	Malonate
Arizona	-	-	-	+
Salmonella	+	+	+	-
Citrobacter	+	-	-	-

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2. Jour. Bact. 71:339. 1956.
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5. Pub. Health Lab. 15:89. 1957.

THE CITROBACTER GROUP

(Including the Bethesda-Ballerup group)

(Third Report)

Citrobacter Definition

The Citrobacter group consists of motile bacteria that conform to the definition of the family Enterobacteriaceae, and have the following biochemical characteristics:

Gas from glucose	+
Lactose	+ or x

[1]Tested in 1.0 per cen peptone solution according to the methods of Kauffmann and Petersen (4).

Sucrose	d
Mannitol	+
Dulcitol	d
Salicin	d
Adonitol	-
Inositol	- or x
Indol	-
Methyl red	+
Voges-Proskauer	-
Ammonium citrate	+
Hydrogen sulfide	+
Urease	-
Gelatin liquefaction	-
Growth in KCN medium	+
Phenylalanine deaminase	-
Sodium malonate	-

In the first report on this group only the slow lactose fermenting cultures were included under the Bethesda group and the Ballerup group (provisional). The second report also was confined to those cultures which did not ferment lactose promptly and the organisms were called the Bethesda-Ballerup group. With the exception of slow fermentation of lactose, the organisms thus listed obviously possess biochemical properties similar to those of the organisms classified in Bergey's Manual (1) as Escherichia freundii and by Werkman and Gillen (2) in the genus Citrobacter. Since there was a marked antigenic continuity between the cultures which fermented lactose rapidly and those which attacked the sugar slowly (West and Edwards, 3) it became obvious that the Bethesda-Ballerup group was only a portion of a larger group previously classified as Escherichia freundii or as Citrobacter. In the interest of clarity of group definitions, the organisms are removed from the Escherichia group and designated as the Citrobacter group.

The members of the Citrobacter group which ferment lactose slowly bear a superficial resemblance to the Salmonella and Arizona groups and often are confused with them. The KCN test of Moeller (4) and the ninhydrin test of Carlquist (5) are of the greatest value in the differentiation of Citrobacter cultures from members of the Salmonella and Arizona

groups (6). Further, organic acids[1] as used by Kauffmann and Petersen (7) and Ellis, Edwards, and Fife (8) also are of help in differentiating the organisms. Typical cultures of the three groups give the following patterns of reaction in these tests:

	Mucate	D-Tartrate	Citrate	Malonate
Salmonella	+	+	+	-
Arizona	-	-	-	+
Citrobacter	+	-	-	-

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8. Public Health Lab. 15:89. 1957.

THE SHIGELLA GROUP

(Third Report)

Shigella Definition

The Shigella group consists of nonmotile bacteria that conform to the definition of the family Enterobacteriaceae and have the following biochemical characteristics:

Gas from glucose	-
Lactose	-
Sucrose	-
Mannitol	d

[1]Tested in 1.0 per cent peptone solution according to the methods of Kauffmann and Petersen (7).

Dulcitol	d
Salicin	-
Adonitol	-
Inositol	-
Indol	d
Methyl red	+
Voges-Proskauer	-
Ammonium citrate	-
Hydrogen sulfide	-
Urease	-
Gelatin liquefaction	-
Growth in KCN medium	-
Phenylalanine deaminase	-
Sodium malonate	-

Some biotypes of Shigella flexneri 6 are known to produce small volumes of gas from glucose and other fermentable substrates. Lactose is not fermented by members of subgroups A, B, or C, but subgroup D cultures generally ferment it after several days' incubation. Sucrose usually is fermented by cultures of subgroup D upon extended incubation but slow production of acid from sucrose also may occur in cultures of certain serotypes of subgroups A, B, and C. Mannitol is not fermented by members of subgroup A but acid usually is produced from this substrate by members of subgroups B, C, and D. Hydrogen sulfide is not produced on Kligler's, triple sugar iron, or lead acetate agars.

The Shigella group is divided into four subgroups. These subgroups are differentiated by means of a combination of the biochemical and serological characteristics of the serotypes contained in each subgroup. Within the subgroups the serotypes are characterized by the possession of a distinctive antigen called the type antigen or main antigen. Within certain serotypes, which possess a common type or main antigen, significant differences occur in the subsidiary or group antigens. The term group antigens as used here refers to the antigens that relate the members of a Shigella subgroup to each other or relate a serotype belonging to one Shigella subgroup to a serotype that belongs to a different Shigella subgroup. Subserotypes have been designated to distinguish the more important of such minor divisions, as in subgroup B (Shigella flexneri). Serotypes in a subgroup are designated by Arabic numerals.

The Shigella schema given in the 1950 Report and the nomenclature and recommendations given in the 1950 and 1953 Reports are reaffirmed. In addition to the recommendations made in the 1950 and 1953 Reports, the following characterizations of the composition of the four subgroups are recommended:

Subgroup A, Shigella dysenteriae, is composed of serotypes that characteristically do not ferment mannitol and which serologically are not significantly related to members of Shigella subgroups B or D. Each serotype possesses a type or main antigen by which it can be differentiates from other shigellae. New Shigella cultures that (a) do not produce acid from mannitol, (b) that may or may not be serologically related to one or more of the serotypes in subgroup A, and (c) that lack significant group antigen relationships to members of subgroups B or D, may be added to subgroup A when the need arises.

Shigella cultures that are closely related to S. dysenteriae 3 and produce acid from mannitol are known. It is recommended that these exceptional cultures be considered as mannitol positive varieties of S. dysenteriae 3 for the present.

Subgroup B, Shigella flexneri, consists of serotypes that usually produce acid from mannitol and which are interrelated by the possession of common group or subsidiary antigens. However, the serotypes each possess a type or main antigen by which they may be differentiated from other shigellae. Serotypes that differ in their group antigen complexes exist in this subgroup. Thus, two cultures that contain the same type or main antigen may differ in the quality and quantity of the antigens present in their group antigen complexes; e.g. subserotypes 1a and 1b.

A qualitative loss variation may occur in certain subgroup B cultures whereby the type or main antigen is lost and the resultant variant cultures contain only group antigen factors. These variants are referred to as X and Y.

Mannitol negative cultures that contain the type antigen of one of the subgroup B serotypes should be classified as mannitol negative varieties of the serotype or subserotype to which they belong. Special designations are not recommended for such varieties.

New serotypes may be added to subgroup B if they possess group antigens in common with known serotypes and if they contain a new type or main antigen demonstrable by appropriate absorption methods. Similarly, new subserotypes may be admitted if they possess a known type or main antigen and contain a new combination of group antigen factors. However, in classification, undue emphasis should not be given to minor variations in the pattern or content of the group or subsidiary antigens of subgroup B cultures.

Subgroup C, Shigella boydii, is composed of microorganisms that ferment mannitol but which serologically are not related to members of subgroup B (or D) in a significant way. The serotypes may be distinguished from other shigellae by identification of their type antigens. Subserotypes are known to exist within some subgroup C serotypes.

Mannitol negative varieties of some subgroup C serotypes are known; these should be designated simply as mannitol negative varieties of the serotypes to which they belong.

Subgroup D, Shigella sonnei, consists of microorganisms that biochemically are similar to members of subgroups B and C but which characteristically ferment lactose upon extended incubation. The existence of mannitol negative members of subgroup D is known, however. Only one serotype is included in subgroup D but new serotypes may be added if they are similar to S. sonnei in their biochemical characters. The O antigens of subgroup D strains are not significantly related to other described shigellea.

The four specific names (Shigella dysenteriae, Shigella flexneri, Shigella boydii, and Shigella sonnei) employed above in connection with Shigella subgroups A, B, C and D, respectively, were conserved by the Judicial Commission as previously recommended by the Shigella Commission and the Enterobacteriaceae Subcommittee (see Opinion 11, approved, and references contained therein, International Bulletin, 4:148-150, 1954).

In the 1953 Subcommittee Report it was recommended that certain described sub judice Shigella serotypes be considered for possible future addition to the Shigella schema. The status of these serotypes, as well as certain others, will be

considered in a supplementary report which will be published at a later date.

Since the purpose for which it was formed was considered to be fulfilled, the ad hoc Shigella Commission, created at the IV International Congress, 1947, was disbanded by majority vote at the Subcommittee meeting held during the VI Congress, Rome, September 6-12, 1953. Matters pertaining to the shigellae are now studied at the International Shigella Centers, by other interested individuals, and by the Subcommittee as a whole.

REFERENCES

Cox, C. D. and Wallace, G. I. 1948. Jour. Immunol. 60:465.
Ewing, W. H., Hucks, M. C., and Taylor, M. W. 1952. Jour. Bact. 63:319.
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Ewing, W. H. 1953. Jour. Bact. 66:333.

SHIGELLA TAXONOMY AND NOMENCLATURE

Serotype	Subserotype		Earlier Designations
Subgroup A			
S. dysenteriae	1		*S. shigae*, Shiga-Kruse bacillus, etc.
	2		*S. schmitzii*, *S. ambigua*.
	3		Q771
	4		Q1167
	5		Q1030
	6		Q454
	7		Q902
	8		Serotype 599-52
Subgroup B			
S. flexneri	1	1a	V
		1b	VZ
	2	2a	W
		2b	WX
	3	3a	Z
		3b	
		3c	
	4	4a	103
		4b	103Z
	5	5	P119
	6	6	Boyd 88, *S. newcastle*, Manchester bacillus
X variant			X
Y variant			Y
Subgroup C			
S. boydii	1		170
	2		P288
	3		D1
	4		P274
	5		P143
	6		D19
	7		Lavington, type T, *S. etousa*
	8		Serotype 112
	9		Serotype 1296/7
	10		Serotype 430
	11		Serotype 34
Subgroup D			
S. sonnei			Sonne-Duval, Sonne III, etc.

THE ESCHERICHIA GROUP

(Third Report)

Escherichia Definition

The Escherichia group consists of motile or nonmotile bacteria that conform to the definition of the family Enterobacteriaceae, and have the following biochemical characteristics:

Gas from glucose	+
Lactose	+ or x
Sucrose	d
Mannitol	+
Dulcitol	d
Salicin	d
Adonitol	-
Inositol	-
Indol	+
Methyl red	+
Voges-Proskauer	-
Ammonium citrate	-
Hydrogen sulfide	-
Urease	-
Gelatin liquefaction	-
Growth in KCN medium	-
Phenylalanine deaminase	-
Sodium malonate	-

Anaerogenic variants are not uncommon.

The contents of the 1950 and 1953 reports on the Escherichia group are reaffirmed.

The differentiation of typical Escherichia cultures from members of other groups of Enterobacteriaceae is not difficult and may be accomplished by utilization of the substrates and tests listed in the definition and in the appended table of biochemical reactions given by Enterobacteriaceae. However, anaerogenic, nonmotile Escherichia biotypes, which do not ferment lactose or do so slowly, occur and such strains often are difficult to distinguish from shigellae. Such biotypes often may be distinguished from members of the Shigella group

by means of Christensen's citrate agar, the ninhydrin test of Carlquist, the lysine decarboxylase test (Moeller), or by their reaction in mucate medium (see Appendix for references to these tests). Members of the Shigella group yield negative reactions in all of these tests, whereas members of the Escherichia group usually give positive results.

Since the second report on the Escherichia group was written the number of O antigen groups has been extended to 137, the number of K antigens to 80, and the number of H antigens to 42. Additional O antigen groups, as well as K and H antigens have been characterized but numbers have not been assigned to them as yet. Considerable progress has been made in studies on the serology of the Escherichia group and many individual serotypes have been characterized within the various O antigen groups. Work on the preparation of an antigen schema embracing the entire Escherichia group, as it is constituted at present, is in progress, but it will be some time before this can be made generally available.

Recommendations regarding methods for the designation of the K antigens of Escherichia serotypes, and for writing the formulas of the serotypes, were given by Kauffmann, Ørskov, and Ewing (1956).

REFERENCES

Ewing, W. H., Tatum, H. W., Davis, B. R., and Reavis, R. W. Studies on the serology of the Escherichia coli group. August 1956. CDC Monograph. Communicable Disease Center, Public Health Service, Atlanta, Ga.

Kauffmann, F., Ørskov, F., and Ewing, W. H. 1956. Internat'l. Bull. Bact. Nomen. Tax. 6:63.

Ørskov, F. 1956. Acta Path. Microbiol. Scand. 39:147.

THE KLEBSIELLA GROUP

(Third Report)

Klebsiella Definition

The Klebsiella group consists of nonmotile, encapsulated bacteria that conform to the definition of the family Entero-

bacteriaceae, and have the following biochemical characteristics:

Gas from glucose	+
Lactose	+
Sucrose	+
Mannitol	+
Dulcitol	d
Salicin	+
Adonitol	+
Inositol	+
Indol	-
Methyl red	-
Voges-Proskauer	+
Ammonium citrate	+
Hydrogen sulfide	-
Urease	(+)
Gelatin liquefaction	-
Growth in KCN medium	+
Phenylalanine deaminase	-
Sodium malonate	+

Since the second report on the Klebsiella group was published, additional biochemical tests not included in the definition have been applied which are helpful in the differentiation of Klebsiella strains from other groups. Hormaeche and Munilla (1) found that Klebsiella cultures produced gas promptly from cellobiose whereas Serratia cultures did not. They also found that Klebsiella cultures produced gas from insoluble starch within 4 days, whereas most Cloaca (Aerobacter) cultures did not. The same authors devised a test for urea hydrolysis which was helpful in the differentiation of Klebsiella and Cloaca (Aerobacter) strains. While Klebsiella cultures hydrolyze urea, their urease activity is much less than that of Proteus cultures, so that the two are not likely to be confused. Moeller (2) demonstrated that decarboxylase tests, particularly the test for ornithine decarboxylase, were useful in the differentiation of Klebsiella and Cloaca (Aerobacter) cultures, the latter giving positive tests for ornithine decarboxylase whereas the former did not. These observations were confirmed by Hormaeche and Munilla (4), Ørskov (3) and Edwards, Fife and Ellis (4). The lysine decarboxylase test is promptly positive with Klebsiella cultures but often negative, weak, or delayed with Cloaca (Aerobacter) strains.

It must be recognized that biochemically aberrant strains occur in the group, the anaerogenic Klebsiella rhinoscleromatis of capsule type 3 being quite unlike the usual strains of Klebsiella pneumoniae in its biochemical properties (Kauffmann, 5). The "ozaenae" strains which occur most frequently in capsule type 4 also possess distinctive biochemical characteristics. While the group is characterized as indol negative and failing to liquefy gelatin, a number of cultures which are indol positive and which liquefy gelatin have been found. In the Klebsiella group there is a rather close correlation between indol production and gelatin liquefaction. Lautrop (6) has pointed out that these indol positive, gelatin positive strains correspond to Bacterium oxytocum of Flügge (1886).

Because of technical difficulties in the recognition of O antigens, the organisms still are classified serologically by capsular reactions and 72 capsule types presently are recognized. Five O antigen groups have been demonstrated (Kauffmann, 5; Ørskov, 7).

REFERENCES

1. Internatl. Bull. Bact. Nomen. Tax. 7:1. 1957.
2. Acta Path. et Microbiol. Scand. 25:259. 1954.
3. Acta Path. et Microbiol. Scand. 40:155. 1957.
4. Unpublished data.
5. Acta Path. et Microbiol. Scand. 26:381. 1949.
6. Acta Path. et Microbiol. Scand. 39:375. 1956.
7. Acta Path. et Microbiol. Scand. 34:145. 1954.

THE CLOACA (AEROBACTER) GROUP

(First Report)

Cloaca (Aerobacter) Definition

The Cloaca (Aerobacter) group consists of motile bacteria that conform to the definition of the family Enterobacteriaceae, and have the following biochemical characteristics:

Gas from glucose	+
Lactose	+
Sucrose	+

Mannitol	+
Dulcitol	d
Salicin	+
Adonitol	d
Inositol	d
Cellobiose	+(with gas formation)
Rhamnose	+
Raffinose	+
Arabinose	+
Xylose	+
Sorbitol	+
Insoluble starch	d(gas not formed, 4 days)
Indol	-
Methyl red	-
Voges-Proskauer	+
Ammonium citrate	+
Hydrogen sulfide	-
Urease	-
Gelatin liquefaction	d
Growth in KCN medium	+
Sodium malonate	d
Phenylalanine deaminase	-
Arginine dihydrolase	d
Lysine decarboxylase	d
Ornithine decarboxylase	+
Glutamic acid decarboxylase	-

Although these biochemical reactions can be considered as typical ,of the group there are aberrant cultures, not fermenting lactose, sucrose, rhamnose, sorbitol, raffinose, MR positive, VP, citrate, or KCN negative. Anaerogenic strains also exist.

Two biochemical types can be differentiated. Type A does not generally ferment inositol nor glycerol, and when so doing does not produce gas in four days; it is arginine positive, lysine negative and liquefies gelatin. Type B promptly ferments with gas inositol and glycerol, is arginine negative, lysine positive and about half of the strains do not liquefy gelatin.

The statement that the group does not split urea is to be understood in the sense that this is so only if special tests for differentiation of Klebsiella and Cloaca (Aerobacter) are used.

Some Klebsialla capsular antigens have been recognized in capsulated strains of the group.

The Starch Test

Beef extract	0.5 gm.
Peptone (Evans)	1.0 gm.
NaCl	0.5 gm.
Water	100 ml.
Phenol red (1/500)	0.6 ml.

Adjust pH at 7.2 - 7.4. Heat at 120° C for 10 minutes, filter, sterilze at 115° C for 20 minutes.

To this basic medium 1 gm. of insoluble starch is added. To avoid lumps and assure uniformity of the medium this substance is finely ground, added to a small amount of cold water, the mixture stirred and then boiled for 5 minutes. The resulting jelly is incorporated into the medium, this is distributed in sterile tubes (120 x 12) fitted with gas bells and sterilized at 110° C for 15 minutes. The tubes are inoculated with a loopful of a 24-hour broth culture and observed daily. If on the fourth day there is no visible gas in the bell insert into the medium the red-hot wire loop, in an effort to determine whether dissolved gas is present.

Phenol-red Solution

Phenol-red	1 gm.
NaOH N/10	40 gms.
Distilled water	460 ml.

The Urea Tests

A) The slow test. (Modification of Christensen medium modified by M. Kristensen)

Bacto peptone	0.1 gm.

NaCl	0.5 gm.
KH_2PO_4	0.2 gm.
Cresol-red (0.4%)	2.4 ml.
Distilled water	100 ml.

Boil all the ingredients, adjust carefully pH to 6.7, filter and sterilize at 115° C for 20 minutes. Add 0.2 gm. urea Seitz filtered or chloroform sterilized. Distribute in sterilized tubes (120 x 12). Inoculate from a 24-hour broth culture and observe for four days, when positive reactions will show a reddish-purple color. Exact adjustment of the medium's reaction is essential, as if it is higher than indicated many Cloaca (Aerobacter) cultures will be positive, and if lower some Klebsialla will be negative.

Cresol-red Solution

Cresol-red	0.1 gm.
NaOH N/50	13.1 ml.
Distilled water	237.0 ml.

B) The quick test

Urea	2.0 gms.
Cresol-red solution (0.4%)	2.0 ml.
Distilled water	100 ml.

Add monopotassium phosphate to a final concentration of 1/10,000, and distribute in hemolysis tubes 1 ml. each. No sterilization is needed. Inoculate with two heavy loopfuls of a 24-hour agar culture and incubate for two hours in the water bath at 45°-50° C. Positive reactions will show a reddish-purple color.

Lactose agar must not be used for growing bacteria for the test, or most Cloaca (Aerobacter) cultures will be positive, and the same happens when some brands of peptone are used. Good results are obtained with agar prepared with beef extract, 0.5 gm., NaCl 0.5 gm., Evans peptone 1 gm., agar 2 gms., for 100 ml. water, and with pH adjusted at 7-7.2.

REFERENCES

Edwards, P.R. and Fife, M.A. Jour. Bact. 70:382. 1955.
Moeller, V. Acta Path. et Microbiol. Scand. 36:158. 1955.
Kauffmann, F. Acta Path. et Microbiol. Scand. 39:85. 1956.
Hormaeche, E. and Munilla, M. Internatl. Bull. Bact. Nomen. Tax. 7:1. 1957.

ADDENDUM

Drs. Edwards, Ewing, and Hormaeche prefer the name, Aerobacter, for the above-mentioned group and recognize at least two subdivisions corresponding to Aerobacter aerogenes and Aerobacter cloacae.

THE HAFNIA GROUP

(Provisional)

Hafnia Definition

The Hafnia group consists of motile bacteria that conform to the definition of the family Enterobacteriaceae, and have the following biochemical characteristics:

	37° C	22° C
Gas from glucose	d	+
Lactose	- or x	- or x
Sucrose	x	x
Mannitol	+	+
Dulcitol	-	-
Salicin	d	d
Adonitol	-	-
Inositol	-	-
Indol	-	-
Methyl red	+	-
Voges-Proskauer	d	+
Ammonium citrate	d	+
Hydrogen sulfide	-	(+)
Urease	-	-
Gelatin liquefaction	-	-
Growth in KCN medium	+	+

Phenylalanine deaminase	-	-
Sodium malonate	d	d

Moeller (1954) gave the name Hafnia to a group of bacteria that were delineated and characterized biochemically during the examination of cultures variously designated as "paracolon aerobacter", "Paracolobactrum aerogenoides", and "intermediate paracolon" bacteria. Subsequently, it was learned that the Hafniae were identical with biotype 32011 of the second division of "paracolon Aerobacter" described by Stuart et al. (1943). Also, Bacillus asiaticus and B. asiaticus, var. mobilis (Castellani, 1906) and B. paratyphi alvei (Bahr, 1919) were found to be Hafnia strains. However, it should be noted that Moeller (1954) mentioned that one culture labeled B. asiaticus var. mobilis was not a member of the Hafnia group.

Members of the Hafnia (32011) group compose a biotype or group of biotypes, which are somewhat similar to other Voges-Proskauer positive Enterobacteriaceae. However, they may be differentiated by the effect of temperature of incubation on their biochemical activities, their failure to ferment lactose or failure to ferment it rapidly, by their failure to ferment sorbitol, by failure to liquefy gelatin, and by other biochemical reactions.

Deacon (1952) and Eveland and Faber (1953) have studied members of the 32011 (Hafnia) biotype and in each of these investigations a number of O and H antigens were established. However, little is known of the antigenic relationship of members of this group of microorganisms to other Voges-Proskauer positive Enterobacteriaceae.

The existence of the biotype or group of biotypes that are named Hafnia herein is well recognized and accepted, but the status of the group itself is undecided at present. That is, the question of whether these bacteria should be considered as a special biotype within an existing group or given group status must be given further study and consideration.

REFERENCES

Deacon, W. E. 1952. Proc. Soc. Expt. Biol. and Med. 81:165.
Eveland, W. C. and Jaber, J. E., Jr. 1953. Jour. Infect. Dis. 93:226.

Moeller, V. 1954. Acta Path. et Microbiol. Scand. 34:145.
Stuart, C. A., Wheeler, K. M., Rustigian, R., and Zimmermann, H. 1943. Jour. Bact. 45:101.

ADDENDUM

Dr. Cowan requested that the following opinion be added to the Hafnia (provisional) Group report.

"Only certain strains labelled B. asiaticus by Castellani belong to the Hafnia group; these are ones distributed in the last few years. Strains received from Castellani in 1924 and 1938 are not members of the Hafnia group.

The important point is that Castellani's description of B. asiaticus does not agree with that of the Hafnia group. In the absence of type strains designated by Castellani at the time he named the organism, his description determines what are the characters of B. asiaticus and a neotype which differed significantly from that description would not be acceptable.

In my opinion B. asiaticus does not enter into any synonymy of the Hafnia group."

THE SERRATIA GROUP

(First Report)

Serratia Definition

The Serratia group consists of motile bacteria that conform to the definition of the family Enterobacteriaceae, some strains produce pink or red pigment and the bacteria have the following biochemical characteristics:

Gas from glucose	d
Lactose	- (or + weak and delayed)
Sucrose	+
Mannitol	+
Dulcitol	-
Salicin	+
Adonitol	d

Inositol	d
Indol	-
Methyl red	-
Voges-Proskauer	+
Ammonium citrate	+
Hydrogen sulfide	-
Urease	-
Gelatin liquefaction	+
Growth in KCN medium	+
Phenylalanine deaminase	-
Sodium malonate	-

When gas is formed the volumes are small, 5 per cent or less. Acid production from lactose within 1 day is uncommon but weak, delayed reactions are often observed. Hydrogen sulfide is not formed on triple sugar iron or lead acetate agars in the usual incubation periods but weak, delayed (5 to 7 days) reactions are obtained with many cultures.

Members of the Serratia group may be separated from Klebsiella on the basis of their motility, failure to utilize malonate, slowness and reluctance to ferment lactose, slight, delayed, or negative urease reactions, and by the small volumes of gas produced by aerogenic cultures. They may be distinguished from members of the Aerobacter (Cloaca) group by their rapid liquefaction of gelatin, reluctance to produce acid from lactose, failure to utilize malonate, and by the small volumes of gas produced from fermentable substrates. The majority of Serratia cultures produced only delayed, slightly acid reactions in cellobiose broth and gas was not formed. Aerobacter (Cloaca) strains generally produce acid and gas from cellobiose within 24 hours' incubation. Serratia strains may be differentiated from those belonging to the Hafnia group by negative methyl red tests at 37° C, by gelatin liquefaction, and by the small volumes of gas produced by aerogenic strains. Sorbitol fermentation also served to distinguish between members of these two groups, since the majority of Serratia strains produced acid from this substrate whereas known members of the Hafnia group did not utilize it. When present, pink or red pigment aided in the differentiation of Serratia cultures.

The above-mentioned comments, as well as the definition of the Serratia group, were based upon results obtained in the examination of 50 strains, all except two of which were isolated during the past few years (Davis, Ewing, and Reavis, 1957). A summary of the biochemical reactions with these

cultures is given in the appended chart. These strains were also employed in the serological studies mentioned below.

In addition to the above-mentioned 50 strains, 57 cultures from various type culture collections were examined (Davis, Reavis, and Ewing, 1957). When received the majority of these cultures was labeled S. marcescens but several were listed under other specific epithets. It should be noted that the same strain was received from two or more type culture collections in many instances and hence there were many duplications among the 57 cultures. Also, many of the strains were very old, having been in the collections for many years. The majority of the cultures gave biochemical reactions similar to those given by the 50 more recently isolated strains mentioned above. However, a few were aberrant in several respects and probably should be excluded from the group. Also, a few cultures, some of which were aerogenic and some of which were anaerogenic, were MR positive and V-P negative. Such cultures were few in number but gave biochemical reactions similar to those given by typical Serratia cultures except for the reversal in the MR and V-P reactions. Therefore, it was obvious that the occasional occurrence of MR positive, V-P negative Serratia must be recognized since a few such cultures were identified in the collection.

Six O antigen groups were established within the Serratia group by Davis and Woodward (1957) and Davis, Ewing, and Reavis (1957) were able to determine the O group of 47 of the 50 more recently isolated strains mentioned above and listed in the summary of biochemical reactions. Several H antigens also were delineated (unpublished data). Preliminary examinations of the O antigens of the 57 cultures from type culture collections indicated that at least 40 belonged to one or another of the six delineated O antigen groups. The O antigen group of several of the cultures could not be determined because of roughness and nine of the undetermined strains were biochemically aberrant.

REFERENCES

Davis, B.R. and Woodruff, J.M. 1957. Canad. J. Microbiol. 3:391.

Davis, B. R., Ewing, W. H., and Reavis, R. W. 1957. Internatl. Bull. Bact. Nomen. Tax. 7:151.

Davis, B. R., Reavis, R. W., and Ewing, W. H. 1957. Unpublished data.

Summary of results of biochemical tests with fifty Serratia group cultures[1]

Test	No. +	No. + delayed	No. -
Glucose	50	0	0
Gas	32	0	18
Lactose	1	29 w	20
Sucrose	50	0	0
Maltose	50	0	0
Mannitol	50	0	0
Dulcitol	0	0	50
Rhamnose	0	0	50
Arabinose	1	0	49
Inositol	31	16	3
Xylose	3	13	31
Raffinose	1	1	48
Sorbitol	48	0	2
Salicin	50	0	0
Adonitol	38	8 w	4
Trehalose	50	0	0
Cellobiose*	4 w	44 w	2
Motility	50	0	0
Simmons' citrate	50	0	0
Christensen's citrate	50	0	0
Urease	0	18 w	32
Indol	0	0	50
Methyl red	0	0	50
Voges-Proskauer	50	0	0
Hydrogen sulfide (TSI)	0	26 w	24
Nitrate reduction	50	0	0
Ammonia glucose agar	50	0	0
Gelatin (22° C)	49	0	1
Phenylalanine deaminase	5 w	0	45
Malonate	0	0	50
Lysine decarboxylation**	49 w	0	1
KCN	48	0	2
Pigment	16	0	34

w - weak; indicates slight reactions.
* - Gas was not formed from cellobiose by any culture.
**- Ninhydrin method of Carlquist.
[1] - From Davis et al. 1957, modified.

THE PROVIDENCE GROUP

(Second Report)

Providence Definition

The Providence group consists of motile bacteria that conform to the definition of the family Enterobacteriaceae, and have the following biochemical characteristics:

Gas from glucose	d
Lactose	-
Sucrose	d
Mannitol	d
Dulcitol	-
Salicin	-
Adonitol	d
Inositol	d
Indol	+
Methyl red	+
Voges-Proskauer	-
Ammonium citrate	+
Hydrogen sulfide	-
Urease	-
Gelatin liquefaction	-
Growth in KCN medium	+
Phenylalanine deaminase	+
Sodium malonate	-

The recommendations and antigenic schema given in the first report on the Providence group (see 1953 Subcommittee Report) are reaffirmed.

Members of the Providence group may be differentiated from members of the Salmonella and Arizona groups on the basis of indol production, fermentation of sucrose and adonitol by many strains, and failure to produce hydrogen sulfide. They may be differentiated from Citrobacter cultures by means of indol production, fermentation of adonitol by many strains, failure to produce acid from lactose, and failure to form hydrogen sulfide. Providence group cultures may be distinguished from members of the Shigella group by means of their utilization of citrate, their motility, and by their fermentation of adonitol, inositol, or sucrose. They are differentiated from

Klebsiella, Aerobacter (Cloaca), Hafnia, and Serratia by their reactions in the MR and V-P tests, by indol production and by failure to ferment lactose. Further, members of the Providence group produce phenylpyruvic acid from phenylalanine, whereas members of the above-mentioned groups do not. Proteus cultures also deaminate phenylalanine to phenylpyruvic acid, but Proteus cultures hydrolyze urea whereas Providence strains do not. Also, members of the Providence group do not liquefy gelatin or produce hydrogen sulfide, whereas two species of Proteus (P. vulgaris and P. mirabilis) do so.

Providence group cultures may be divided into two principal biogroups, each of which is further divisible into a number of biotypes (Ewing, Tanner, and Dennard, 1954). The biogroups may be differentiated and the biotypes that occurred most commonly may be allocated to the appropriate biogroup on the basis of the following:

	Biogroup 1	Biogroup 2
Gas from glucose	+	-
Adonitol	+	-
Inositol	-	+

Since the antigenic schema contained in the first report on the Providence group was written the number of O antigen groups has been extended to 62, the number of H antigens to 30, and the number of known serotypes to 156.

As mentioned in the introduction to this Report, it is recommended that the Providence group be maintained as a separate entity until international agreement is reached regarding the taxonomy and nomenclature of the Proteus and Providence groups.

REFERENCE

Ewing, W. H., Tanner, K. E., and Dennard, D. A. 1954. Jour. Infect. Dis., 94:134.

RECOMMENDED BIOCHEMICAL METHODS FOR GROUP DIFFERENTIATION WITHIN THE ENTEROBACTERIACEAE

	Recommended Test No.	Alternate Test No.
Gas from glucose	1	2
Lactose	1	2
Sucrose	1	2
Mannitol	1	2
Dulcitol	1	2
Salicin	1	2
Adonitol	1	2
Inositol	1	2
Indol	3	4
Methyl Red	5	
Voges-Proskauer	6	7
Ammonium Citrate	8	
Hydrogen Sulfide	9	10
Urease	11	12
Gelatin	13	14
KCN	15	
Motility	16	17
Phenylalanine	18	19
Malonate	20	

N.B.

It will be noted that the temperature of incubation recommended for the majority of the tests is 37° C. However, if preliminary examinations indicate that a culture may be a member of the Hafnia group, tests should be repeated and incubated at 20° to 22° C.

Test No. 1

Broth base for carbohydrates: Liebig's meat extract broth, pH 7.4.

Meat extract	5 gms.
Peptone	10 gms.
Sodium chloride	3 gms.
Dibasic sodium phosphate	2 gms.
Indicator solution	12 ml.
Distilled water	1000 ml.

Liebig's, Lemco, Difco, or other comparable meat extract and Orthana, Bacto, Evans, or other similar peptone may be used.

Indicator solution

Bromthymol blue	1 gm.
1/10 Normal NaOH	25 ml.
Distilled water	475 ml.

Add 0.5 per cent of test substance, tube with inverted insert tubes, and sterilize at 121° C for 15 minutes. Glucose, mannitol, adonitol, inositol, and salicin may be added to the medium prior to sterilization. Disaccharides such as lactose and sucrose should be sterilized by filtration and added to previously sterilized basal medium.

Inoculation: Inoculate lightly from a young (16-20 hrs. incubation) agar slant culture.

Incubation: 37° C. Negative tests should be observed for 30 days.

Test No. 2

Fermentation broth base. Alternate method for carbohydrate tests.

Peptone	10 gms.
Meat extract	3 gms.
Sodium chloride	5 gms.
Andrade's indicator	10 ml.
Distilled water	1000 ml.

Liebig's, Lemco, Difco, or other comparable meat extract and Orthana, Bacto, Evans, or other similar peptone may be used.

Andrade's Indicator

Distilled water	100 ml.
Acid fuchsin	0.5 gm.
Sodium hydroxide (1N)	16 ml.

The fuchsin is dissolved in the distilled water and the sodium hydroxide is added. If, after several hours, the fuchsin is not sufficiently decolorized add an additional 1 or 2 ml. of alkali. The dye content of different samples of acid fuchsin varies quite widely and the amount of alkali which should be used with any particular sample usually is specified on the label. The reagent improves somewhat on aging and should be prepared in sufficiently large amount to last for several years. The indicator is used in amounts of 10 ml. per liter of medium.

Adjust reaction to pH 7.1-7.2. Tube with inverted insert tubes and sterilize in autoclave at 15 lbs. for 15 minutes. Fermentable substances may be sterilized by filtration of 5 or 10 per cent solution and added aseptically to the base in sufficient amount to make a final concentration of 0.5 or 1.0 per cent.

Inoculation and incubation: as given under Test No. 1.

Test No. 3

Medium for indol tests. Peptone water.

Peptone	20 gms.
Sodium chloride	5 gms.
Distilled water	1000 ml.

Leave reaction unadjusted and sterilize at 121° C, 15 minutes. Two per cent Bacto peptone may be used or, if desired other comparable peptones such as Evans, Merck, or Vaillant No. 5. One per cent tryptone also is satisfactory.

Inoculation: Inoculate lightly from a young agar slant culture.

Incubation: 37° C, 40 to 48 hours. Test with Kovacs' reagent. Add about 0.5 ml. of reagent, shake tube gently. A deep red color develops in the presence of indol.

Kovacs' reagent

Pure Amyl or isoamyl alcohol	150 ml.
Paradimethylaminobenzaldehyde	10 gms.
Concentrated pure hydrochloric acid	50 ml.

Dissolve aldehyde in alcohol and then slowly add acid. The dry aldehyde should be light in color. Kovacs' reagent should be prepared in small quantities and stored in the refrigerator when not in use.

Remarks on indol test (applicable to both Test No. 3 and No. 4). Tests for indol production may be made after 24 hours' incubation, but if this is to be done, one or two ml. of culture should be removed aseptically from the tube and the test made on this sample. If the test is negative, the remaining portion of the peptone water culture should be reincubated for an additional 24 hours.

Test No. 4

Medium for indol tests. Alternate method.

Casein digest broth	1000 ml.
Tap water	2000 ml.

Sterilize at 121° C, 15 minutes.

The casein digest broth is prepared in the manner given by Kristensen et al., Brit. J. Exper. Path. 6:291. 1925.

(A medium prepared with 1.5 per cent Trypticase (BBL), Casitone (Difco) or other commercially available product that gives results comparable to those obtained with the medium listed in test No. 3 may be used. If a casein digest is used it should be an enzymatic digest - not an acid one).

Inoculation and Incubation: as given in Test No. 3. Test with Ehrlich-Boehme reagent. Carefully add about 0.5 ml. of reagent. Do not shake tube. A rose purple color develops in the presence of indol.

Ehrlich-Boehme reagent.

Paradimethylaminobenzaldehyde	8 gms.
95 to 96 per cent ethyl alcohol	760 ml.
Concentrated pure hydrochloric acid	160 ml.

Test No. 5

The Methyl Red test.

Buffered peptone glucose broth. Three modifications of the Clark and Lubs formula are listed below. Any of these may be used for both the Methyl Red and Voges-Proskauer tests. It is understood that 0.5 per cent Evans peptone may be substituted in the formulas.

A. Dibasic potassium phosphate	0.5 gm.
Peptone, Orthana special	0.5 gm.
Glucose	0.5 gm.
Distilled water	100 ml.

Mix dibasic potassium phosphate, peptone, and distilled water, heat to boiling and then filter. Sterilize at 121° C, 15 minutes. Add 0.5 gm. of glucose. Tube and boil for 10 minutes.

B. MR-VP medium (BBL)	
Dipotassium phosphate	5.0 gms.
Polypeptone	7.0 gms.
Glucose	5.0 gms.
Distilled water	1000 ml.

Suspend ingredients in water and heat slightly to dissolve them. Tube and sterilize at 118° C to 121° C for 15 minutes.

C. MR-VP medium (Difco)	
Buffered peptone	7 gms.
Glucose	5 gms.
Dipotassium phosphate	5 gms.
Distilled water	1000 ml.

Inoculation: Inoculate lightly from a young agar slant culture.

Incubation: 37° C, for 48 hours to 5 days. Tests should not be performed with cultures incubated less than 48 hours but 48 hrs. is sufficient for the majority of cultures. However, if the results of tests made at 48 hrs. are equivocal, the tests should be repeated with cultures that have been incubated for 4 or 5 days.

Test reagent:

Methyl Red	0.1 gm.
Ethyl alcohol (95 to 96%)	300 ml.

Dissolve dye in the alcohol and then add sufficient distilled water to make 500 ml. Use 5 or 6 drops of reagent per 5 ml. of culture. Reactions are read immediately. Positive tests are bright red, weakly positive tests are red-orange and negative tests are yellow.

Test No. 6

Voges-Proskauer test. Test for the production of acetylmethylcarbinol (acetoin) and 2,3 - butyleneglycol.

Buffered peptone glucose broth. The same medium used in Test No. 5, may be used for the V-P test.

Inoculation: as in Test No. 5.

Incubation: 37° C, 48 hrs.

Test reagent (O'Meara)

Potassium hydroxide	40 gms.
Creatine	0.3 gm.
Distilled water	100 ml.

Dissolve the alkali in distilled water and add creatine. Use the reagent in the proportion of 0.1 ml. per 1.0 ml. of culture. Place tubes in a water bath (about 48° to 50° C) for 2 hrs. or at 37° C for 4 hrs. Aerate by shaking the tubes. A positive V-P test is indicated by the development of a delicate eosin-pink color.

Test No. 7

Voges-Proskauer test. Alternate method.

Buffered peptone glucose broth, as used in Test No. 5 or 6.

Inoculation: same as Test Nos. 5 and 6.

Incubation: 37° C, 4 days.

Test reagent (Barritt)

One ml. of 6 per cent alcohol solution of alphanapthol and 0.4 ml. of 40 per cent potassium hydroxide are added to 2 ml. of broth culture. Positive reactions occur at once or within a few minutes. The Barritt test is somewhat more sensitive than the O'Meara method.

Test No. 8

Ammonium citrate medium. Test for the utilization of sodium citrate and ammonium salts.

Sodium chloride	5.0 gms.
Magnesium sulfate	0.2 gm.
Ammonium dihydrogen phosphate	1.0 gm.
Dipotassium phosphate	1.0 gm.
Sodium citrate	5.0 gms.
Agar (washed vigorously for 3 days)	20.0 gms.

Add 40 ml. of 1:500 bromthymol blue indicator solution. (See Test No. 1)

Sterilize at 121° C, 15 minutes and slant so as to obtain a 1 inch butt and 1-1/2 inch slant.

Inoculation: Prepare a saline suspension from a young agar slant culture and inoculate the slant of the medium with a straight wire from the saline suspension. If desired, the butt of the medium may be stabbed.

Incubation: 37° C for 4 days.

The above-mentioned medium is available from several commercial sources under the name of Simmons' citrate agar. These preparations are quite satisfactory.

Test No. 9

Hydrogen sulfide production

Ferrous chloride gelatin

Liebig's meat extract	7.5 gms.
Peptone (Parke-Davis or Evans)	25 gms.
Sodium chloride	5 gms.
Gelatin	120 gms.
Ferrous chloride (10 per cent solution)	5 ml.
Distilled water	1000 ml.

The gelatin medium is boiled and the freshly prepared 10 per cent solution of ferrous chloride immediately added. The medium is tubed in narrow tubes and the tubes are placed in cold water to cool. The tubes are sealed with cork stoppers that have been soaked in hot paraffin.

Inoculation: Inoculate by making a stab into the depths of the medium column.

Incubation: 20° C. Observe daily for at least 7 days for blackening caused by hydrogen sulfide production.

(This same medium is used for the determination of gelatin liquefaction and hence is actually observed for a much longer period. See Test No. 13).

Test No. 10

Hydrogen sulfide production. Alternate method.

Triple sugar iron agar (a modification of Kligler's iron agar).

Beef extract	3 gms.
Yeast extract	3 gms.
Bacto peptone	15 gms.
Proteose peptone, Difco	5 gms.
Lactose	10 gms.
Sucrose	10 gms.

Glucose	1 gm.
Ferrous sulfate	0.2 gm.
Sodium chloride	5 gms.
Sodium thiosulfate	0.3 gm.
Agar	12 gms.
Phenol red	0.024 gm.
Distilled water	1000 ml.

Polypeptone (BBL) or Evans peptone may be substituted for the Bacto peptone and proteose peptone in the formula given above.

After sterilization at 121° C, 15 minutes, the medium is slanted with a deep butt (1 inch butt, 1.5 inch slant).

Inoculation: The butt of the medium is stabbed and the slant is streaked.

Incubation: 37° C, observe daily for 5 to 7 days for blackening caused by hydrogen sulfide production.

Remarks about tests for hydrogen sulfide production. If highly sensitive methods are used, hydrogen sulfide production can be demonstrated in cultures that belong to most groups of Enterobacteriaceae and it seems probable that all Enterobacteriaceae produce varying quantities of hydrogen sulfide from inorganic or organic substances. However, for a hydrogen sulfide test to be of value in group differentiation within the Enterobacteriaceae it must be poised at a certain definite level of sensitivity. The media listed above (Tests Nos. 9 and 10) are poised at such a level and thus may be used for group differentiation. Although methods which utilize lead acetate paper strips suspended over peptone water cultures may be of value in intragroup differentiation within certain groups, these methods are of no value in intergroup differentiation and cannot be recommended for the latter purpose.

Test No. 11

Test for production of urease.

Christensen's urea agar

Peptone	1 gm.
Sodium chloride	5 gms.

Glucose	1 gm.
Monobasic potassium phosphate	2 gms.
Phenol red	0.012 gm. (6 ml. of 1:500 solution)
Agar	20 gms.
Distilled water	1000 ml.

Adjust to pH 6.8 to 6.9

Bacto, Evans, or other comparable peptone may be used. After sterilization at 121° C for 15 minutes, the basal medium is cooled at 50-55° C and then sufficient 20 per cent urea solution (Seitz filtered) is added to give a final concentration of 2 per cent. Then the medium is slanted with a deep butt. This medium may be employed in fluid form if desired, by omitting the agar.

Inoculation: The medium is inoculated heavily over the entire surface of the slant.

Incubation: 37° C. Examine at 2 hrs., 4 hrs., and after overnight incubation. Negative tubes should be observed daily for 4 days. Urease positive cultures produce an alkaline reaction in the medium evidenced by a red color.

Test No. 12

Rapid urease test. Alternate method.

Yeast extract	0.1 gm.
Monobasic potassium phosphate	0.091 gm.
Dibasic sodium phosphate	0.095 gm.
Urea	20 gms.
Phenol red	0.01 gm.
Distilled water	1000 ml.

This medium is Seitz filtered and tubed in sterile tubes in 3 ml. amounts. The basal medium may be made up in 900 ml. of distilled water and sterilized at 121° C, 15 minutes. After cooling 100 ml. of 20 per cent urea solution is added and the medium tubed in sterile tubes in 3 ml. amounts.

Inoculation: Three loopfuls (2 mm. loop) from an agar slant culture are inoculated into a tube of medium and the tube is shaken to suspend the bacteria.

Incubation: Tests are incubated in a water bath at 37° C and the results are read after 10 minutes, 60 minutes, and 2 hrs.

Test No. 13

Gelatin liquefaction

The same tube of ferrous chloride gelatin medium used for the determination of hydrogen sulfide production (Test No. 9) may be observed for gelatin liquefaction. Tubes should be incubated at 20° C for 30 days.

Test No. 14

Gelatin liquefaction. Alternate method.

Nutrient gelatin

Beef extract	3 gms.
Peptone	5 gms.
Gelatin	120 gms.
Distilled water .	1000 ml.

Polypeptone (BBL), Bacto, Evans, Parke-Davis, or other comparable peptone may be used. Sterilize at 121° C 12 minutes.

Inoculation: Inoculate by stabbing the medium with a wire, using inoculum from an agar slant culture.

Incubation: 20° C, 30 days.

Test No. 15

Potassium cyanide (KCN) test. Test for growth in the presence of KCN.

Peptone, Orthana special	10 gms.
Sodium chloride	5 gms.
Monobasic potassium phosphate	0.225 gm.
Dibasic sodium phosphate	5.64 gms.
Distilled water	1000 ml.
Adjust pH to 7.6	

The basal medium is sterilized at 121° C, 15 minutes, then refrigerated until thoroughly chilled. To the cold medium is added 15 ml. of 0.5 per cent KCN solution (0.5 gms. KCN dissolved in 100 ml. sterile distilled water). The medium is then tubed in approximately 1.0 ml. amounts in sterile tubes (12 x 150 mm. or 10 x 100 mm.) and stoppered quickly with corks sterilized by heating in paraffin. The medium in such tubes can be stored safely for 2 weeks at 4° C.

It has been found that 0.3 per cent Bacto Proteose Peptone No. 3 or 0.3 per cent Evans Peptone may be substituted for Orthana Peptone.

Inoculation: The tubes are inoculated with 1 loopful of a 24 hr. broth culture grown at 37° C.

Incubation: Tests are incubated at 37° C and observed daily for 2 days. Positive results are indicated by growth in the presence of KCN.

Test No. 16

Motility test. Medium.

Beef extract	3 gms.
Peptone	10 gms.
Sodium chloride	5 gms.
Agar	2.5 gms.
Distilled water	1000 ml.

Any good peptone may be used. Adjust reaction to pH 7.4 and tube, about 8 ml. per tube, and sterilize at 121° C, 15 minutes.

Inoculate by stabbing into the top of the column of medium to a depth of about 5 mm.

Incubation: 37° C for 2 days. If negative, follow with further incubation at 21 to 25° C for 5 days.

Test No. 17

Motility test. Alternate method.

Peptone	10 gms.
Meat extract	5 gms.
NaCl	3 gms.
Kobe agar	2.25 gms.
Distilled water	1000 ml.

Any good peptone and meat extract may be employed. Adjust reaction to pH 7.4. Sterilization, inoculation, and incubation are the same as that given for Test No. 16.

Test No. 18

Phenylalanine agar. Test for deamination of phenylalanine to phenylpyruvic acid.

Yeast extract	3 gms.
DL-phenylalanine	2 gms.
(or L-phenylalanine	1 gm.)
Disodium phosphate	1 gm.
Sodium chloride	5 gms.
Agar	12 gms.
Distilled water	1000 ml.

Tube and sterilize at 121° C for 10 minutes and allow to solidify in a slanted position (long slant).

Test reagent: 10 per cent (w/v) solution of ferric chloride.

Inoculation: Inoculate the slant of the PA agar with a fairly heavy inoculum from an agar slant culture.

Incubation: 4 hrs. or, if desired 18 to 24 hrs. at 37° C.

Following incubation, 4 or 5 drops of ferric chloride reagent are allowed to run down over the growth on the slant. If phenylpyruvic acid has been formed a green color develops in the syneresis fluid and in the slant.

Test No. 19

Phenylalanine. Alternate method.

Inoculate the whole surface of an agar slant. Incubate overnight; if growth is good, suspend in 0.5 ml. saline. Occasional strains (though rarely Proteus strains), give insufficient growth, in which case the growth from several slants should be used. The suspension should contain about 50,000 million organisms or more, but need not be standardized. Transfer the suspension to a test tube and add 0.5 ml. of 0.2 per cent DL-phenylalanine in saline (or 0.1 per cent L-phenylalanine), preserved with 0.01 per cent merthiolate. Add 1 drop of 0.01 per cent phenol red and make alkaline with M/10 Na_2CO_3. Shake vigorously and place tubes nearly flat on a table. Leave for 4 hours and add 10 per cent H_2SO_4 dropwise until the color changes through yellow to pink. Add sufficient $(NH_4)_2SO_4$ to saturate the solution and then 4 or 5 drops of half-saturated $FeNH_4(SO_4)_2$. Shake well and read results when the green color is at a maximum or, if negative, after about 1 minute.

Positive reactions are graded as + (faint greenish color), ++ (distinct, but weak or only moderately strong green color), and +++ (dark green to bluish-green color).

Henriksen, S. D. Jour. Bact. 60:225-231. 1950.

Test No. 20

Sodium malonate broth. Test for utilization of malonate.

Yeast extract	1 gm.
Ammonium sulfate	2 gms.
Dipotassium phosphate	0.6 gm.
Monopotassium phosphate	0.4 gm.
Sodium chloride	2 gms.
Sodium malonate	3 gms.
Bromthymol blue	0.025 gm.
Distilled water	1000 ml.

Sterilize at 121° C for 15 minutes.

Inoculation: Inoculate from a young agar slant culture.

Incubation: 37° C for 48 hours.

Positive results are indicated by a change in the color of the indicator from green to Prussian blue.

Additional Biochemical Tests

There are a number of additional tests and methods that are of considerable value in the differentiation of strains that belong to certain groups of Enterobacteriaceae. These are listed below:

The Ninhydrin Test of Carlquist (1956). This test is of particular value in the differentiation of members of the Salmonella and Arizona groups on the one hand, from Citrobacter (including Bethesda-Ballerup) strains on the other. The majority of cultures that belong to the Salmonella and Arizona groups give positive tests while, with few exceptions, Citrobacter strains are negative (Edwards, Fife, and Ewing, 1956). The test is also of value in the differentiation of shigellae and Escherichia cultures of the Alkalescens-Dispar biotypes. The shigellae are negative when tested by the ninhydrin method of Carlquist, whereas the majority of Alkalescens-Dispar cultures are positive.

The amino acid decarboxylase tests of Moeller (1954a, 1954b, 1955). These include tests for decarboxylation of lysine, arginine, ornithine, and glutamic acid, all of which are of value in taxonomic studies in the Enterobacteriaceae. It should be noted that within certain groups e.g. the Cloaca (Aerobacter) group the results obtained with the lysine decarboxylase test described by Moeller (1954b) and the ninhydrin method of Carlquist (1956) may not be comparable. Hence, it is very important to designate the method employed, particularly in published reports.

Christensen's Citrate Agar (1949). Test for citrate utilization in the presence of organic nitrogen. This method is of particular value in the differentiation of shigellae and Escherichia cultures of the Alkalescens-Dispar biotypes (Edwards, Fife, and Ewing, 1956).

Organic Acid Tests. The organic acid tests, L-tartrate, D-tartrate, M-tartrate, sodium citrate, and mucate, are also of considerable value, particularly in certain areas within the Enterobacteriaceae (see Kauffmann and Petersen, 1956).

Reduction of nitrates. Although the nitrate reduction test is of little or no value for group differentiation within the Enterobacteriaceae, it is included because a negative test is often of value in the exclusion of otherwise doubtful strains. The results of negative nitrate tests should always be confirmed. This may be done by adding a minute amount of zinc dust to the tube. The development of a red color indicates the presence of unreduced nitrate.

Potassium nitrate (nitrite free)	0.2 gm.
Peptone	5.0 gms.
Distilled water	1000 ml.

Tube in 5 ml. amounts and sterilize at 121° C, for 15 minutes on each of three successive days. After inoculation from an agar slant culture the medium is incubated at 37° C, for 4 days.

Test reagents

A. Dissolve 8.0 grams of sulfanilic acid in 1000 ml. of 5 normal acetic acid.

B. Dissolve 5.0 grams of alphanaphthylamine in 1000 ml. of 5 normal acetic acid.

Immediately before use equal parts of solutions A and B are mixed and 0.1 ml. of the mixture is added to each culture. Positive tests for reduction of nitrate to nitrite are indicated by the development of a red color within a few minutes.

Nitrate reduction. Alternate method.

Tryptone	5 gms.
Neopeptone	5 gms.
Agar	2.5 gms.
Distilled water	1000 ml.

Boil and adjust pH to 7.3-7.4 then add

KNO_3 (nitrite free)	1.0 gm.
Glucose	0.1 gm.

Sterilization: 121° C, 15 minutes.

Inoculation: The medium may be inoculated by stabbing into the column of semisolid agar medium.

Incubation: 37° C, 24 hrs. The occasional culture that gives apparently equivocal results should be retested after 1, 2, 3, and 4 days' incubation.

Test reagents

The sulfanilic acid and alphanaphthylamine reagents given above (A and B) are used in the same manner.

References for Additional Tests

Carlquist, P. R. 1956. Jour. Bact. 71:339-341.
Edwards, P. R., Fife, M. A., and Ewing, W. H. 1956. Amer. Jour. Med. Technol. 22:28-35.
Kauffmann, F. and Petersen, A. 1956. Acta Path. Microbiol. Scand. 38:481-491.
Moeller, V. 1954a. Acta Path. Microbiol. Scand. 34:102-114.
1954b. ibid. 35:259-277.
1955. ibid. 36:158-172.

GROUP DIFFERENTIATION OF ENTEROBACTERIACEAE BY BIOCHEMICAL TESTS
(REACTIONS OF TYPICAL CULTURES OBTAINED WITH RECOMMENDED METHODS)

GROUP	Salmonella	Arizona	Citrobacter **	Shigella	Escherichia	Klebsiella	Aerobacter or Cloaca	Hafnia		Serratia	Proteus				Providence
								37° C	22° C		vulgaris	mirabilis	morganii	rettgeri	
Gas from Glucose	+	+	+	-*	+	+	+	d	+	d	+	+	+	d	d
Lactose	-	+ or x	+ or x	-*	+ or x	+	+ or x	- or x	- or x	- or x	-	-	-	-	-
Sucrose	-	-	d	-*	d	+	+	x	x	+	+	x	d	d	d
Mannitol	+	+	+	d	+	+	+	+	+	+	-	-	-	+	d
Dulcitol	d	-	d	d	d	d	d	-	-	-	-	-	-	-	-
Salicin	-	-	d	-	d	+	+	d	d	+	+	d	-	d	-
Adonitol	-	-	-	-	-	+	d	-	-	d	-	-	-	+	d
Inositol	d	-	- or x	-	-	+	d	-	-	d	-	-	-	+	d
Indol	-	-	-	d	+	-	-	-	-	-	+	-	+	+	+
Methyl Red	+	+	+	+	+	-	-	+	-	-	+	+	+	+	+
Voges-Proskauer	-	-	-	-	-	+	+	d	+	+	-	d	-	-	-
Ammonium citrate	+	+	+	-	-	+	+	d	+	+	d	d	-	+	+
Hydrogen sulfide	+	+	+	-	-	-	-	-	(+)	-	+	+	-	-	-
Urease	-	-	-	-	-	(+)	d	-	-	-	+	+	+	+	-
Gelatin	-	(+)	-	-	-	-	d	-	-	+	+	+	-	-	-
KCN	-	-	+	-	-	+	+	+	+	+	+	+	+	+	+
Motility	+	+	+	-	+	-	+	d	+	+	+	+	+	+	+
Phenylalanine deaminase	-	-	-	-	-	-	-	-	-	-	+	+	+	+	+
Malonate	-	+	-	-	-	+	d	d	d	-	-	-	-	-	-

+ Positive 1 or 2 days.
x Late and irregularly positive.
- Negative.
d Different biochemical types.

(+) Gelatin liquefied slowly, urea hydrolysed slowly, or hydrogen sulfide produced slowly and in small amounts.
* Shigella sonnei ferments lactose and sucrose slowly. Certain biotypes of S. flexneri 6 produce gas.
** Citrobacter group, including Bethesda-Ballerup.

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REPORT OF THE LEPTOSPIRA SUBCOMMITTEE TO THE INTERNATIONAL COMMITTEE ON BACTERIOLOGICAL NOMENCLATURE

J. C. Broom, Secretary
Leptospira Subcommittee

At a meeting of the Subcommittee held in 1953 during the Sixth International Congress for Microbiology it was agreed that the serological characteristics of strains of leptospires provides the best basis for classification available at present. These characteristics are determined by agglutination-lysis and agglutinin-absorption tests, so the Subcommittee has been engaged in studying the techniques used by different workers in an attempt to obtain agreement on standardized methods. In this work the Subcommittee has received the collaboration of the Leptospira Study Group of WHO/FAO. A meeting of the Group, which included eight members of the Subcommittee, was convened at Amsterdam in November 1955, by the Secretary, Dr. M. M. Kaplan. At that meeting agreement was reached on what were considered to be satisfactory methods of carrying out these serological tests, and the conclusions were published as World Health Organization Technical Report Series No. 113 entitled "Diagnosis and Typing in Leptospirosis".

As was mentioned in the Minutes of the last meeting, the Subcommittee were in favour, in theory, of the preparation and issue of Standard Agglutinating Antisera. In practice, however, financial considerations would probably create difficulties. Through the good offices of WHO arrangements have been made for small quantities of dried antisera suitable for preliminary trials to be prepared by the Army Medical Service Graduate School, Washington, D. C., U.S.A.; the Istituto Superiore di Sanita, Rome, Italy; and the National Institute of Health, Tokyo, Japan.

One of the activities undertaken by the Subcommittee was the establishment of "Libraries of Reprints" in a number of countries (now eleven in all) at Institutes specially interested in leptospiral investigations. Reprints of articles are obtained by the Secretary as soon as possible after publication, and are distributed to the Libraries where they can be consulted by any interested worker. During the last four years copies of nearly 300 articles have been added to the libraries.

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CONSERVATION OF THE FAMILY NAME ENTEROBACTERIACEAE, OF THE NAME OF THE TYPE GENUS, AND DESIGNATION OF THE TYPE SPECIES

Opinion No. 15

Judicial Commission of the International
Committee on Bacteriological Nomenclature

The Editorial Board of the Judicial Commission published a statement (1) relative to a proposal that the family name Enterobacteriaceae Rahn 1937 be conserved, that the type genus be designated, that the name of the type genus be conserved, and that the type species be designated. The Commissioners were requested to vote by ballot on the several proposals.

The result of the vote authorizes the Editorial Board acting for the Judicial Commission to publish the following OPINION. This OPINION is final unless revoked or modified by action of the International Committee on Bacteriological Nomenclature.

Opinion No. 15

1. The family name Enterobacteriaceae Rahn 1937 (p. 280) is placed in the list of conserved family names (nomina conservanda familiarum).

2. The genus Escherichia Castellani and Chalmers 1919 (p. 941) is designated as the type genus of the family Enterobacteriaceae Castellani and Chalmers 1919.

3. The generic name Escherichia Castellani and Chalmers 1919 (p. 941) is placed in the list of conserved generic names (nomina conservanda generum).

4. The type species of the genus Escherichia Castellani and Chalmers 1919 (p. 941) is Escherichia coli (Migula) Castellani and Chalmers 1919 (p. 941) (basonym Bacillus coli Migula 1895 (p. 27); hyponym Bacterium coli commune Escherich 1885 (p. 518).

REFERENCES

1. Editorial Board. Proposal to conserve the family name Enterobacteriaceae and to designate the type genus. Internatl. Bull. Bact. Nomen. and Taxon. 7(4):165. 1957.
2. Minutes of the meeting of the International Committee on Bacteriological Nomenclature held at Rome in connection with the VI International Congress for Microbiology, September, 1953. Meeting of the 7th September. Internatl. Bull. Bact. Nomen. and Taxon. 3(4):159. 1953.